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UNIVERSITY OF ALBERTA

BLACK SPRUCE FROM PEATLAND AND UPLAND HABITATS IN ALBERTA: POPULATION VARIATION AND DIFFERENTIATION

BY

ZHANG MING WANG

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

DEPARTMENT OF FOREST SCIENCE

EDMONTON, ALBERTA

FALL 1991

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled BLACK SPRUCE FROM PEATLAND AND UPLAND HABITATS IN ALBERTA: POPULATION VARIATION AND DIFFERENTIATION submitted by ZHANG MING WANG in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOHPY in FORESTRY.

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J. Hoddinott

for W. H. Parker

Date: 17 Sept. 1991

TO MY PARENTS, SISTERS, AND BROTHERS

TO MY WIFE, GONG ZHANG, AND MY SON, LONG

Abstract

Seeds were collected from 3 pairs of peatland and adjacent upland black spruce (Picea mariana (Mill.) B.S.P.) populations at 3 sites to study ecotypic differentiation in black spruce in Alberta, Canada. The study consisted of 3 parts. In an allozyme study, 40 maternal trees/population were genotyped using 16 enzymes coded by 28 loci. Mean number of alleles/locus, percent of polymorphic loci, observed and expected heterozygosities, F_{st}, X^2-test, genetic identity, and discriminant analysis were used in data analyses. In a germination study, seeds were germinated at 0, -0.5, -1.0, or -1.5 MPa by 15, 25, or 35°C for 30 days. Data were analyzed by ANOVA. In a morphological and physiological study of seedlings, two greenhouse experiments were conducted. In the first, seedlings were grown at 2 light irridiances (100 or 20% of full light) by 3 moisture conditions (wet, medium, or dry) for 120 days. In the second, seedlings were grown at 2 substrate temperatures (ambient or cooled) by 2 nutrient regimes (with or without fertilization) for 130 days. Variables measured were seedling height, dry weight (top, root, total, and top/root), net assimilation, stomatal and mesophyll conductances to CO_2, and water use efficiency. For both experiments, data for plasticity and genetic variation within populations were generated. Data were analyzed by ANOVA and discriminant analysis.

In the allozyme study, there was little genetic differentiation between the habitats. In the germination and greenhouse studies, seeds from upland trees germinated better and their seedlings grew faster, but seeds from different habitats had no adaptive responses to the treatments. Genetic variation within populations was similar between habitats. However, peatland populations had higher plasticity than upland ones, and there were significant site-dependent habitat differences for plasticity and other traits. Among sites, the populations from site 3 were the most different. Within habitats, upland populations were more variable in the allozyme study, but in the other studies, peatland ones were. Results from all three studies showed that peatland and upland black spruce was not well differentiated. Different sets of traits showed different variation patterns among populations, indicating different characters are controlled by different genes or gene complexes.

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Chapter 1. General introduction

1.1. Literature review

The species

Black spruce (*Picea mariana* (Mill.) B.S.P.) is one of the most abundant conifers in North America where it is found from 41 to 68°N and 53 to 161°W (Fowells 1965). It is one of Canada's most important pulpwood species (Fowler and Mullin 1977). The climatic conditions in the natural range of black spruce vary from place to place in precipitation (annual mean: 10 to 55 inches), length of frost-free season (60 to 140 days), and temperatures (minimum and maximum: -60 and 40°C).

Black spruce forms stands on a variety of soils ranging from well drained upland sandy or rocky soils to water-logged peatland organic soils (Fowells 1965). It grows in pure stands or in mixtures of various species including white spruce, balsam fir, jack pine, lodgepole pine, white birch, tamarack, and trembling aspen. Black spruce is the only conifer species, besides tamarack, which can thrive in the cold microclimate, unfavorable soil conditions, and high water table of peatland in western Canada (Moss 1953).

Black spruce has a shallow root system without a taproot. Trees begin seed production as early as 10 years of age. Black spruce is a fire adapted species (Black and Bliss 1980) with persistent and semi-serotinous cones which retain viable seeds for several years (Fowells 1965). In Alberta, both peatland and upland stands of black spruce are of fire origin (Horton and Lees 1961). Black spruce is commonly classed as shade tolerant. Its seedlings will develop in the understorey with as little as 10% of full light, but development is much superior in the open (Place 1955).

The habitats

Uplands are well-drained sites with mineral soil, whereas peatlands are water-logged ecosystems in which at least 30 cm of peat have accumulated (Shotyk 1988). Compared with uplands, peatlands are characterized by a high water table, poor soil aeration, low nutrient content, and cold soils (Payandeh 1973). In western Canada, peatland black spruce is often found in mixture with tamarack. The stands remain open long after sexual maturity, and the trees are stunted and rarely reach merchantable size (Lieffers 1986). The poor growth is due to the stressful abiotic environment. Black spruce trees grow much better on upland than on peatland sites; but, on upland stands, they are usually overtopped by white spruce, trembling aspen, and balsam poplar. Here, competition for light aboveground and for moisture and nutrients belowground may significantly affect survival and growth of black spruce. Thus, the abiotic and biotic environments of black spruce are very different between

peatland and upland habitats.

Population differentiation in general

There are two major kinds of population variation patterns, clinal and ecotypic. Clinal variation (Huxley 1938) is defined as a gradation in measurable characters of organisms. Ecotypic variation (Turesson 1922) is characterized by an abrupt pattern change resulting from disruptive selection where habitats are discontinuous. Edaphic variation is one kind of ecotypic variation with special reference to soil conditions.

Natural populations tend to differentiate over time into subpopulations because of selection, random drift, founder effects, and restricted gene flow. Large population size, longevity, outcrossing mating system, and extensive gene flow reduce or prevent differentiation among populations. Depending on the geographical distribution pattern, the total ecological amplitude of a species, and the kind and intensity of selection pressure, both clinal and ecotypic differentiation can develop within a species.

In forest tree species, population differentiation among geographically distant populations has proved a common phenomenon in terms of morphological traits (e.g., Fowler et al. 1988, Rehfeldt 1988, Loopstra and Adams 1989), allozyme frequencies (e.g., Cheliak et al. 1988, Schuster et al. 1989, Lagercrantz and Ryman 1990), and physiological traits (e.g., Farmer and Reinholt 1986, Rehfeldt 1988). Localized (over small distances) population differentiation has also been detected based on morphological traits (e.g., Hall 1955, Squillace and Bingham 1958, Youngman 1965, Hermann and Lavender 1968, Rehfeldt 1988), physiological traits (Fryer and Ledig 1972, Ledig and Korbobo 1983), and allozyme frequencies (Mitton et al. 1977, Knowles 1980, Linhart et al. 1981, Knowles 1984, Mitton et al. 1989). Evidence of edaphic population differentiation has been reported, too, with respect to allozyme frequencies (Furnier and Adams 1986, Millar 1989, Mitton et al. 1989) and morphology (McMillan 1956, Habeck 1958, Kruckeberg 1967, Teich and Holst 1974, Musselman et al. 1975, Jenkinson 1977).

Levels of genic variation noted in allozyme surveys are generally consistent with estimates of genetic variation for morphological traits in trees (Guries 1984). However, population differentiation detected at allozyme loci is often far less than that found for morphological and physiological traits (e.g., Cheliak et al. 1988, Farmer et al. 1988, Loopstra and Adams 1989, Moran and Adams 1989, Yeh et al. 1986). It has been shown that variability in one character set is not a good predictor of that in another (Linhart et al. 1989). While the reasons for the lack of concordance among different sets of traits are not clear, it suggests that neither type of study alone is sufficient to describe the genetic structure of a species.

Population differentiation in black spruce

Population differentiation in black spruce has been well studied, and both clinal and ecotypic differentiation have been found. Morgenstern (1969a, b) sampled seeds from a range-wide selection of populations in Canada, but mainly from Ontario, and studied 13 characters in seed germination, phenology, survival, and growth of seedlings. He found that the variation was clinal among the populations in relation to regional climates. Dietrichson (1969) used the same black spruce populations as Morgenstern (1969a, b) and reported essentially the same clinal pattern of variation with respect to early growth and hardiness of seedlings.

Fowler and Park (1982) studied 99 provenances of black spruce distributed throughout the species range, but mainly from Ontario and the Maritimes. Their seedlings were planted in 10 test locations in the Maritimes Region. They found that the variation pattern was clinal in terms of seed weight and seedling characteristics (nursery performance, and 5-year survival and height after planting).

In Newfoundland, a study of variation in cone length and diameter, and phenology and growth of progenies among black spruce populations showed ecotypic differentiation which was attributed to distinct regional climates (Khalil 1975). However, the variation in height growth (Hall 1986) and in allozyme frequencies (Yeh et al. 1986) for black spruce in Newfoundland have been found to be both clinal and ecotypic.

Segaran (1979) studied 20 local provenances of black spruce in Manitoba. He found the variation pattern to be essentially clinal. In central New Brunswick, variation of allozyme frequencies among black spruce populations was not significant (Boyle and Morgenstern 1987).

To study ecotypic differentiation in black spruce, comparisons have often been made between peatland and upland populations. In this regard, variable results have been reported. Fowler and Mullin (1977) studied seed size, germination, and seedling survival and growth of black spruce from northern Ontario. They did not find peatland-upland ecotypes. Parker et al. (1983) studied morphological and chemical variation in black spruce in northwestern Ontario and found little ecotypic differentiation between the habitats. However, their results indicated that the peatland trees were more variable for cone, flavonoid, and allozyme characters. Boyle et al. (1990) studied one pair of peatland and upland black spruce populations in Ontario and found that they were similar in allozyme frequencies but different in inbreeding coefficients.

On the other hand, O'Reilly et al. (1985) found some allozyme differentiation between peatland and upland black spruce populations in northern Ontario and greater variation among upland than among peatland populations. Differentiation between peatland and upland black spruce in Ontario has been shown for seed weight and germination (Trueland 1980),

and root growth (Fung 1975, Trueland 1980, Lee 1984). Thus, evidence on ecotypic differentiation between peatland and upland black spruce is inconclusive in the east of Canada.

Black spruce is well established in both peatland and upland habitats in western Canada, but its population differentiation has not been well studied. Sproule (1988) studied one peatland and one upland black spruce stand (20 km apart) in Alberta. He found no difference in outcrossing rates between them.

Methods used in the study of population differentiation

Most ecological genetic studies use 1), provenance/progeny tests with analyses of morphological and/or physiological variation, usually carried out under common garden or greenhouse conditions, and/or 2), gel electrophoresis: detection of variability at allozyme loci. Some use morphological traits and/or secondary plant products, such as terpenes and flavonoids, in natural populations. However, these are of limited use in population genetic studies because the genetic basis of the variation in the secondary compounds is poorly understood (Hamrick et al. 1981) and the morphologial phenotypes in natural habitats are the effects of genotype by environment interaction.

Phenotypes of quantitative traits are subject to marked ontogenetic and environmental influences (Yeh and Arnott 1986). Compared to allozyme phenotypes, their adaptive values are readily interpretable, but their inheritance is unknown. Allozymes, on the other hand, are largely the products of single genes (Conkle 1972). They are inherited according to simple genetic principles and are relatively easy to assay (Fins and Seeb 1986). They are under relatively less environmental control than other quantitative traits (Ayala 1976). Individuals can be easily genotyped, and the data obtained provide direct measures of genetic variation within and among populations. Compared to other approaches, enzyme electrophoresis is less expensive and produces results more rapidly (Yeh and Arnott 1986). Despite several shortcomings, such as nonspecificity of enzyme assays, environmental modification of mobility, restrictive detection of base substitutions, and biases in the choice of loci, the allozyme technique is the best method currently available for a direct evaluation of the genetic resources of a species (Brown and Moran 1981).

Studies of population differentiation have contrasted the use of allozyme variation with morphological variation (Schwaegerle et al. 1986). Some have found a relationship between these two measures of genetic differentiation, but others have not. Morphological traits usually exhibit a greater degree of differentiation among populations than allozyme polymorphisms (Wheeler and Guries 1982). Lewontin (1984) demonstrated that it should be more difficult to detect differences among populations using data on allele

frequencies than on metric traits. Mitton (1983) speculated that different evolutionary forces are acting upon these two different sets of characters. Different sets of traits provide information about different facets of the same genome (Linhart et al. 1989). Thus, variable results from different study approaches should be viewed as complementary rather than contradictory to each other, and different approaches should be combined together to examine variation of the same populations.

1.2. Objectives

This project was designed to study population differentiation in black spruce between peatland and upland habitats in Alberta. It consisted of 3 major parts: part one was to investigate differences in allozyme frequences; part two was to study seed germination ecology; and part three was to examine seedling morphological and physiological responses to various controlled environments.

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Chapter 2. Allozyme variation

2.1. Introduction

Allozymes are protein variants controlled by allelic variants at a single gene locus and easily detectable by gel electrophoresis. Their genetic inheritance can be easily demonstrated. Most allozyme loci are codominant, and gene frequecies can be calculated without genetic crosses (Hamrick et al. 1981). There is some evidence that natural selection is related to differentiation of allozymes among populations within species and among species (Nevo et al. 1988) although allozyme variation may be selectively neutral (Kimura 1983).

Since the early 1970's, allozyme techniques have been used in genetic studies of forest tree populations (Hamrick et al. 1981). They are especially useful in gymnosperms because the haploid maternal gametophyte can be readily sampled. Thus, individuals can be genotyped easily and data provide direct measures of genetic variation within and among populations (Brown and Moran 1981).

Allozyme studies have shown that forest trees are among the most variable of all organisms studied to date (Hamrick et al. 1981). These high levels of variability have provided good opportunities for examining the organization of genetic variation within and among populations (Guries 1984). Studies have shown that genetic differentiation not only occurs among geographically separated populations (e.g., Cheliak et al. 1988, Schuster et al. 1989, Lagercrantz and Ryman 1990), or localized ones (Mitton et al. 1977, Knowles 1980, Mitton et al. 1989), but also among breeding units within populations of forest trees (Linhart et al. 1981, Knowles 1984). However, only a small proportion of the total genetic diversity (often less than 10%) is found among populations (Merkle and Adams 1987). This pattern is considered to be a consequence of the extensive inter-population gene flow and long life span of trees (Loveless and Hamrick 1984) and the difficulty of detecting population differentiation in allozymes (Lewontin 1984), or allozymes are neutral to selection.

Allozyme techniques have been used to study black spruce population differentiation. Yeh et al. (1986) found that differentiation among 21 populations in Newfoundland was both clinal and ecotypic with respect to regional and altitudinal environments. Boyle and Morgenstern (1987) studied 6 populations in central New Brunswick and detected little significant differentiation among them with 99% of the variation being within populations.

Using allozymes, edaphic ecotypes have been detected in Engelmann spruce from dry and wet sites (Mitton et al. 1989), in Jeffrey pine in response to ultramafic soils (Furnier and Adams 1986), and in bishop pine associated with pygmy-forest soil (Millar 1989). Peatland and upland

habitats are very different in terms of nutrient availability, and soil structure, temperature, moisture, pH, and aeration (Bradbury and Grace 1983), and light condition for black spruce development. These differences in environment might result in variable selection pressures such that plant species which grow in both habitats may evolve edaphic ecotypes.

To investigate ecotypic differentiation in black spruce between peatland and upland habitats, Parker et al. (1983) sampled trees from 3 sites (well-drained upland to wet peatland) in northwestern Ontario and found that the populations from different sites had similar allozyme frequencies, but peatland trees were more variable. O'Reilly et al. (1985) studied 5 pairs of peatland and upland populations in northern Ontario. They found that peatland populations had higher genetic variability (percent of polymorphic loci and mean heterozygosity), but were less differentiated from one another than upland populations. Boyle et al. (1990) studied one pair of peatland and upland populations in Ontario. They reported that the populations were similar in allozyme frequencies, but the upland trees were more inbred. Thus, evidence of black spruce population differentiation between peatland and upland habitats in the east is still inconclusive.

Black spruce is abundant on peatland and upland sites in western Canada, but the genetic structure of populations and the extent of ecotypic differentiation have not been well studied. Sproule (1988) found no differences in outcrossing between one peatland and one upland black spruce stand (20 km apart) in Alberta. My study was designed to examine the population differentiation in black spruce from peatland and upland habitats in Alberta by allozyme electrophoresis.

2.2. Materials and methods

Three pairs of peatland and upland populations were selected in Alberta (Table 2-1). Sites 1 and 2 were about 25 km apart and 120 km away from site 3. Peatland and upland populations at each site are about 1 km apart. Within each of the 6 populations, 40 black spruce trees were selected for collection of cones. They were dominant or codominant, and at least 40 m apart from each other. Average stand height, diameter at 1.5 m height, and age were estimated from the first 15 trees in each stand. Trees were aged by counting the rings on discs cut from the stem base. Stand density was estimated by making total counts of all trees (taller than 1 m) in a 25m x 25m plot. These statistics are summarized in Table 2-1.

More than 50 one-year-old cones were collected from the upper portion of the crown of each tree in June, 1989. Cones were stored at 3°C from 1 to 4 weeks and then dried at 50°C for 24 hours. Seeds were extracted by shaking dried cones, then dewinged, cleaned, and stored by seed lot at 3°C.

Table 2-1. Sites and stand statistics for the 6 black spruce populations (Ht-height; dbh-diameter at 1.5 m Ht)

Site	Habitat	Species	Trees per ha	Age	Ht (m)	dbh (cm)
1. 55 08'N 114 15'W	peatland	black spruce	1875	42	4.15	5.84
		tamarack	2250			
	upland	black spruce	325	81	15.90	20.80
		white spruce	416			
		aspen	240			
2. 55 02'N 114 02'W	peatland	black spruce	4176	41	4.47	5.37
		tamarack	528			
	upland	black spruce	1056	55	12.37	17.90
		white spruce	112			
		tamarack	16			
3. 53 25'N 116 01'W	peatland	black spruce	1648	71	5.8	7.95
		tamarack	2250			
	upland	black spruce	592	91	16.62	21.00
		white spruce	528			
		tamarack	144			

Seeds from the 40 sample trees in each stand were germinated at 25°C for 5 days. Haploid megagametophytes were used for electrophoresis. The extraction buffer contained 0.1M tris; 0.2M sucrose, 2% PEG 8000, 0.6% PVP, 1M EDTA, 0.1% BSA, 0.002M ascorbic acid, and 0.66% mercaptoethanol. The horizontal electrophoresis gels (22cm x 7cm x 1.2cm) contained 12.5% starch. Banding patterns of 16 enzyme systems were studied by three buffer systems. A tris-citrate/lithium-borate buffer system (pH 8.1) (Ridgeway et al. 1970) was used to analyze glutamate dehydrogenase (GDH), shikimate dehydrogenase (SKD), colorimetric esterase (CE), leucine-aminopeptidase (LAP), and malic enzyme (ME). The system was run at 300 v and 4°C for about 2.5 hours. A histidine-tris (pH 7.0) buffer system (Cheliak and Pitel 1984) was used to analyze 6-phosphogluconic dehydrogenase (6PG), acid phosphatase (APH), aspartate aminotransferase (AAT), phosphoglucose isomerase (PGI), and glucose-6-phosphate dehydrogenase (G6P). Finally, a continuous tris-citrate buffer system (0.028M citric acid, 0.13M tris, pH 7.0) was used to analyze malate dehydrogenase (MDH), fluorescent esterase (FLE), isocitrate dehydrogenase (IDH), adenylate kinase (AK), phosphoglucomutase (PGM), and aconitase (ACO). The last two systems were run at 200 v and 4°C for about 3

hours. Staining techniques and procedures were similar to those of Yeh and O'Malley (1980). Additional staining solutions were: AK: 100 ml Tris (Trizma base, 0.2M, pH 8.0), 2 ml 1% NADP, 1 ml 10% MgCl, 2 ml 1% PMS, 2 ml 1% MTT, 100 units of G-6PGH, 100 mg glucose, 100 mg ADP, 7 mg hexokinase; APH: 300 mg Na-alpha-napthyl acid phosphate, 150 mg Fast garnet GBC salt, 90 ml Na acetate (0.2M, pH 5.0), 2 ml 10% MgCl; FLE: 10 mg 4-methylumbelliferyl acetate, 24 ml Na acetate (0.2M, pH 5.0); LAP: 100 ml Tris/Maleic buffer (Tris 0.2M and Maleic acid 0.2M, pH 5.3), 2 ml 10% MgCl, 100 mg L-leucyl-beta-napthylamide, 140 mg Fast black K Salt; and SKD: 80 ml Tris (0.2M, pH 8.0), 0.60 g agar, 2 ml 1% MTT, 2 ml 1% PMS, 2 ml 1% NADP, and 4 mg shikimic acid.

Eight haploid megagametophytes per parent tree were assayed individually to determine parental genotypes. Megagametophytes from trees that were homozygous at most loci were run on each gel as standards. When interpreting the electrophoretic banding patterns, multiple loci for a given enzyme system were designated as 1 for the most anodal zone, and 2, the next, etc. The most common allele of a locus was designated as 0, the faster moving ones, as 1, 2, etc., and the slower, as -1, -2, etc.

Data analysis was done by both univariate and multivariate methods. Heterogeneity X^2-tests (Workman and Niswander 1970) were used to detect differences in allele frequencies among the 6 populations, 3 sites, populations within habitats, and between habitats. BIOSYS-I (Swofford and Selander 1981) was used to calculate the mean number of alleles per locus based on all the loci, percentage of polymorphic loci (frequency of most common allele $\leq$ 0.99), F-statistics (Wright 1965), genetic identity (Nei 1978), and average expected heterozygosity based on Hardy-Weinberg expectation. In multivariate analyses, the genotypes of individual trees were transformed to allozyme profiles according to Yeh et al. (1985). Three discriminant analyses (using SPSS-PC, Norusis 1988) were used to assess genetic differentiation of the 6 individual populations, 3 sites, and 2 habitats.

2.3. Results

Allele Frequencies and Genetic Variability: The 16 enzymes gave 28 electrophoretically demonstrable loci. Of these, 20 were polymorphic and yielded a total of 51 alleles. The 8 monomorphic loci were (Aat-3, Aph-1, G6p-1, Me-1, Pgi-2, Pgi-3, Pgm-1, 6pg-1). The allele frequencies of the 6 populations are summarized in Table 2-2.

The mean number of alleles per locus based on all the loci was very similar among the 6 populations, 3 sites, and between the 2 habitats (Table 2-3). Overall, the peatland populations had fewer polymorphic loci than upland ones (Table 2-3). Sites 1 and 2 had an identical percentage of polymorphic loci, which was lower than that of site 3.

Table 2-2. Allele frequencies of the polymorphic loci for the 6 populations (The frequencies of the least frequent allele at each locus are not shown)

Locus	Number of Alleles per locus	Allele	Peatland Site 1	Peatland Site 2	Peatland Site 3	Upland Site 1	Upland Site 2	Upland Site 3
Aat-1	2	1	.975	1.000	.975	.975	.975	.925
Aat-2	2	0	1.000	1.000	.988	.988	.988	.950
Aco-1	4	0	.613	.762	.625	.625	.525	.650
		1	.213	.125	.287	.275	.250	.213
		2	.162	.112	.087	.100	.225	.138
Ak-1	2	0	1.000	1.000	.988	1.000	1.000	1.000
Ak-2	2	0	.813	.800	.775	.788	.813	.788
Aph-2	2	0	.988	.988	1.000	1.000	1.000	1.000
Ce-1	2	0	1.000	1.000	1.000	1.000	1.000	.988
Fle-1	2	0	.925	.938	.887	.962	.950	.850
Gdh-1	2	0	.725	.738	.750	.800	.675	.700
Idh-1	2	0	1.000	1.000	.988	1.000	1.000	1.000
Idh-2	3	0	.450	.438	.538	.300	.438	.550
		1	.550	.550	.463	.700	.563	.438
Lap-1	2	1	.538	.550	.538	.550	.512	.500
Mdh-1	4	0	.962	.950	.950	.962	.962	.938
		-1	.025	.038	.038	.025	.025	.063
		2	.013	.000	.013	.013	.013	.000
Mdh-2	3	0	.975	.988	.913	.962	.950	.913
		1	.025	.013	.075	.038	.050	.075
Mdh-3	3	0	.863	.800	.875	.863	.788	.887
		1	.112	.125	.063	.087	.150	.050
Me-2	4	0	.887	.850	.850	.887	.813	.875
		1	.112	.112	.125	.100	.162	.112
		2	.000	.025	.025	.013	.025	.013
Pgi-1	2	0	.988	1.000	1.000	1.000	.988	1.000
Pgm-2	3	0	.325	.275	.350	.325	.425	.363
		1	.663	.712	.637	.663	.575	.637
		2	.013	.013	.013	.013	.000	.000
Skd-1	4	0	.637	.650	.625	.675	.688	.712
		-1	.175	.188	.200	.188	.125	.150
		1	.138	.162	.150	.125	.175	.125
6pg-2	3	1	.663	.712	.675	.625	.663	.738
		2	.313	.250	.275	.313	.287	.213

Table 2-3. Genetic variability at 28 loci (standard errors)

Data Source		Mean number of alleles per locus[1/]	% of polymorphic loci[2/]	Mean heterozygosity observed	Mean heterozygosity expected[3/]
Peatland	1	1.9 (0.2)	57.1	0.152 (0.039)	0.163 (0.040)
	2	1.8 (0.2)	50.0	0.148 (0.037)	0.156 (0.038)
	3	1.9 (0.2)	60.7	0.161 (0.039)	0.173 (0.039)
Upland	1	1.8 (0.2)	53.6	0.158 (0.040)	0.155 (0.038)
	2	1.8 (0.2)	57.1	0.160 (0.040)	0.174 (0.041)
	3	1.9 (0.2)	57.1	0.162 (0.039)	0.174 (0.038)
Mean		1.85	55.93	0.157	0.166
Habitat					
Peatland		2.1 (0.2)	60.9	0.154 (0.037)	0.164 (0.039)
Upland		2.0 (0.2)	67.7	0.160 (0.039)	0.169 (0.039)
Site	1	1.9 (0.2)	60.7	0.155 (0.039)	0.159 (0.039)
	2	2.0 (0.2)	60.7	0.154 (0.038)	0.166 (0.040)
	3	2.0 (0.2)	64.3	0.161 (0.038)	0.173 (0.038)

[1/] Calculated on the basis of all loci.
[2/] Frequency of the most common allele $\leq$ 0.99.
[3/] Unbiased estimate (Nei 1978) based on Hardy-Weinberg expectation.

The observed frequency of heterozygotes was generally less than that expected under Hardy-Weinberg, indicating a deficiency of heterozygotes in these natural populations (Table 2-3). But, the 2 estimates of heterozygosity were not significantly different. The mean heterozygosities (observed and expected) were similar among the 6 populations, 3 sites, and between the 2 habitats. Overall, the upland populations had greater observed and expected heterozygosities. Site 3 had greater observed and expected heterozygosities than the other 2 sites. There was a greater variation in observed heterozygosities among peatland than among upland populations. There was equal variability in expected heterozygosities among populations for both habitat types.

Overall, there was very little differentiation for allozyme frequencies among the 6 populations, 3 sites, populations within habitats, or between habitats based on X^2-tests, F_{st}-values, and genetic identities (Table 2-4). The X^2-test only showed significant ($p \leq 0.05$) differentiation among the 6 populations at 1 locus, the 3 sites or the 3 upland populations at 2 loci (Table 2-4). Only a very small proportion of the variation was attributable to differences among populations ($F_{st}=0.01$), among sites ($F_{st}=0.005$), or between habitats ($F_{st}=0.001$). The data indicated greater differentiation among sites ($F_{st}=0.005$, $I=0.9996$) than between habitat types ($F_{st}=0.001$, $I=1.0$) and among upland ($F_{st}=0.012$, $I=0.9989$) than among peatland ($F_{st}=0.005$, $I=1.0$) populations although these differences may not be significant. The values of F_{is} (over all loci) were positive indicating a deficiency of heterozygotes within populations (Table 2-4).

Discriminant Analysis of Population Differentiation: The discriminant analyses also indicated greater isozyme differentiation among sites than between habitats and that site 3 was the most divergent. However, no discriminant function could significantly ($p \leq 0.05$) separate the 6 populations, 2 habitats, or 3 sites (Table 2-5).

The discriminant analysis for the 6 populations illustrated a complex pattern of differentiation. The discrimination was not significant with only about 39% of the trees correctly classified (Table 2-5). The first discriminant function (DF1) accounted for about 40% of the total variation, of which about 25% was due to differences among populations (e.g., r^2, canonical correlation squared). DF2 accounted for about 22% of the total variation, of which about 16% was due to the differences among populations (Table 2-5). Peatland populations at different sites did not have consistent scoring patterns compared to their corresponding upland populations, and the peatland and upland population groups were not well separated (Fig. 2-1). DF1 seems to reflect the geographic separation of the three sites with populations from sites 1 and 2 having smaller

scores than those from site 3.

Table 2-4. Results of analysis of allozyme variability, A: hierarchical heterogeneity X^2-tests (df in brackets), B: values of F_{st} and F_{is}, and C: mean genetic identities[1/]

Locus	Among 6 populations	between 2 habitats	among 3 peatlands	among 3 uplands	among 3 sites
A.					
Aat-1	8.02 (5)	2.65 (1)	2.03 (2)	3.34 (2)	4.12 (2)
Aat-2	8.67 (5)	3.62 (1)	2.09 (2)	2.96 (2)	4.56 (2)
Aco-1	18.33 (15)	3.64 (3)	8.64 (6)	6.06 (6)	5.73 (6)
Ak-1	3.13 (5)	1.04 (1)	2.09 (2)	0.00 (2)	2.08 (3)
Ak-2	0.56 (5)	0.00 (1)	0.35 (2)	0.20 (2)	0.33 (2)
Aph-1	3.14 (5)	2.09 (1)	1.05 (2)	0.00 (2)	1.04 (2)
Ce-1	3.13 (5)	1.04 (1)	0.00 (2)	2.09 (2)	2.08 (2)
Fle-1	9.74 (5)	0.03 (1)	1.43 (2)	8.28 (2)*	8.02 (2)*
Gdh-1	3.74 (5)	0.10 (1)	0.13 (2)	3.51 (2)	1.33 (2)
Idh-2	3.13 (5)	1.04 (1)	2.09 (2)	0.00 (2)	2.08 (2)
Idh-3	17.66 (5)*	1.03 (1)	3.86 (2)	12.77 (2)*	10.75 (2)*
Lap-1	0.68 (5)	0.21 (1)	0.03 (2)	0.44 (2)	0.20 (2)
Mdh-1	7.61 (5)	1.09 (3)	3.39 (6)	3.12 (6)	4.20 (6)
Mdh-2	10.94 (10)	0.76 (2)	6.94 (4)	3.24 (4)	8.90 (4)
Mdh-3	9.02 (10)	0.06 (2)	4.02 (4)	4.93 (4)	7.75 (4)
Me-1	7.49 (15)	1.11 (3)	4.24 (6)	2.13 (6)	4.71 (6)
Pgi-1	4.17 (5)	0.00 (1)	2.09 (2)	2.09 (2)	1.04 (2)
Pgm-2	7.24 (10)	2.45 (2)	1.08 (4)	3.70 (4)	0.87 (4)
Skd-1	8.67 (15)	2.28 (3)	4.33 (6)	2.07 (6)	3.84 (6)
6pg-1	4.70 (10)	0.77 (2)	1.39 (4)	2.54 (4)	1.99 (4)
Total	139 (160)	25 (32)	51 (64)	63 (64)	75 (64)
B. F_{st}	0.010	0.001	0.005	0.012	0.005
F_{is}	0.044	0.053	0.053	0.036	0.049
C. Mean genetic identity	0.9998	1.0000	1.0000	0.9989	0.9996

[1/] Frequencies of all the alleles of the polymorphic loci were used in the analyses.
* X^2 value is significant at 5% level.

Table 2-5. Results of discriminant analysis for the data of allozyme profiles (DF-discriminant function)

Data Source	DF	% of variance	Canonical r	p value	Mean % of grouped cases correctly classified
6 populations	1	40.20	.4855	.7228	38.75
	2	21.73	.3780	.9875	
	3	17.02	.3398	.9958	
	4	11.19	.2811	.9972	
	5	9.86	.2652	.9755	
3 sites	1	70.36	.4544	.2310	53.75
	2	29.64	.3143	.8788	
2 habitats	1	100.00	.3207	.8722	61.67

Within sites, habitat differentiation was greatest at site 2, and smallest at site 1 (Fig. 2-1). DF1 and DF2 were both important in separating the habitats at sites 2 and 3. At site 2 the peatland population had a smaller score than the upland on DF2, while at site 3 the opposite was true.

In the discriminant analysis comparing the 3 sites only about 54% of the trees were correctly classified (Table 2-5). Sites 1 and 2 separated from site 3 on DF1 (Fig. 2-1) which accounted for about 70% of the total variation (Table 2-5). About 21% of this was due to differences among sites. Sites 1 and 2 separated on DF2 while site 3 had an intermediate value for this function (Fig. 2-1).

Results of the third discriminant analysis showed that only about 9% of the variation was accounted for by the differences between habitats (Table 2-5). Although about 62% of the trees were correctly classified (Table 2-5), the separation was not significant (Table 2-5).

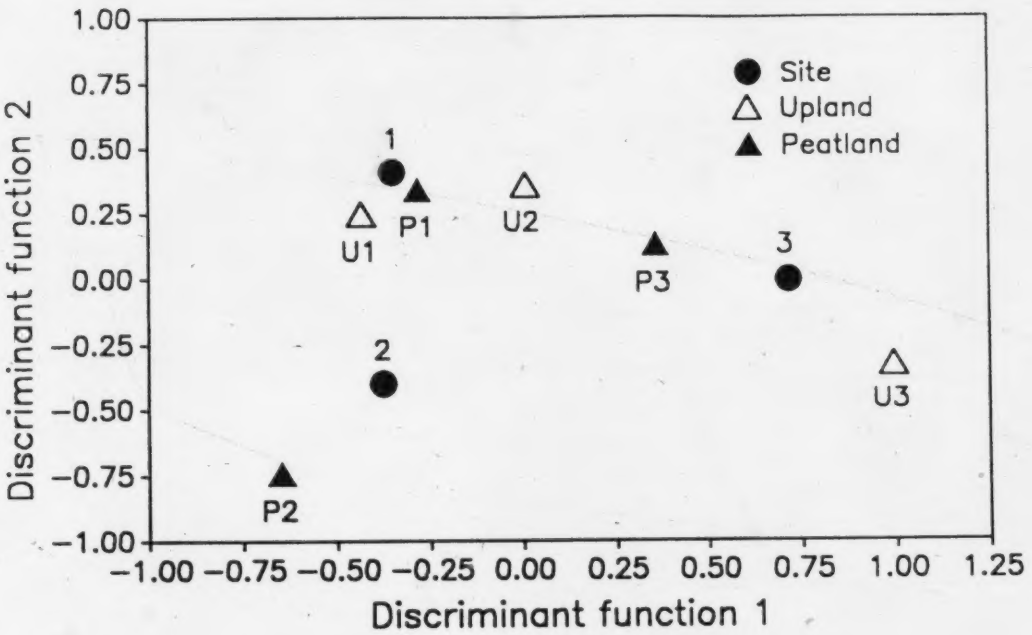

Fig. 2-1. Discriminant plot for the 6 black spruce populations at 3 sites based on allozyme profile data. One population contained 1320 cases (40 trees x 33 allele codings in the allozyme profile). One site consisted of 2 populations.

2.4. Discussion

Levels of genetic variation of black spruce in this study are comparable to those of other studies of conifers and black spruce (Table 2-6). However, they are higher than the mean values obtained by pooling the values of the studies listed in Table 2-6 and lower than the mean values of 20 conifer species (Hamrick et al. 1981) (Table 2-6). A negative relationship may exist between the number of enzyme loci surveyed and the magnitude of various measures of population variability (Hamrick et al. 1979). As there are more species, more studies, and more loci included in the studies listed in Table 2-6, it is believed that the mean

estimates in Table 2-6 better represent the mean genetic variability of conifers. Thus, the genetic variability of the black spruce populations in this study is approximately the same as the average levels in other studies of conifers Table 2-6).

My results indicate that there was a heterozygote deficiency in the populations studied. This contrasts to the heterozygote excess in black spruce in Yeh et al. (1986). Heterozygote deficiency has been previously found in other species including Pinus contorta (Dancik and Yeh 1983, Knowles 1984), and Larix occidentalis (Fins and Seeb 1986). Many outbreeding plant species show heterozygote deficiency (Brown 1979). Restricted distribution and predominant selfing can be excluded as factors affecting the amount and pattern of genetic variability in black spruce. Small scale population genetic substructuring is likely in conifers as a result of differentiation (Mitton et al. 1977, Linhart et al. 1981, Knowles 1984) or the natural patterns of seed dispersal and seedling establishment (Libby et al. 1969). Sampling of trees from such substructured populations may result in heterozygote deficiency (Wahlund 1928, Dancik and Yeh 1983), lower measures of genetic variability, and higher population differentiation.

In this study, upland and peatland populations generally had similar levels of genetic variability. The differences in percent of polymorphic loci and heterozygosities between habitats varied with sites; and, overall, upland populations had higher genetic variability than peatland populations. This is in contrast to the results of Parker et al. (1983), and O'Reilly et al. (1985) who found higher variability in peatland than in upland populations of black spruce. While the reason for this discrepancy is not clear, smaller samples of trees (25 or 10 per population) and enzymes (4 or 10) in the above 2 studies respectively than there were in my study (16 enzymes and 40 trees per population) might have contributed to it.

There was an indication of greater differentiation among upland than peatland populations. This result is in agreement with O'Reilly et al. (1985). This could be due to environmental conditions being more uniform among peatland than among upland populations. Greater variation among upland sites could result in greater differentiation among upland populations.

Environmental differences between upland and peatland habitats may be expected to lead to development of edaphic ecotypes in black spruce (O'Reilly et al. 1985). However, there was little genetic differentiation in allozyme frequencies between the 2 habitats in this study. This result agrees with those reported by Parker et al. (1983) and Boyle et al. (1990). However, O'Reilly et al. (1985) found some allozyme differences between the 2 habitats for the same species although it is not known if the differences

were significant.

It was found that the majority of genetic variability was intrapopulational and that population differentiation was related to geographic distance. This is an indication of differentiation due to reduced gene exchange and genetic drift (Kimura and Weiss 1964), or founder effects, or natural selection. Still, only 0.5% of the genetic variation was due to differences among sites, and the mean genetic distance among sites was very small (0.0004) (Table 2-6). Comparing with other studies (Table 2-6), these values are among the smallest reported. Thus, if geographic separation has caused genetic differentiation in black spruce in Alberta, such differentiation is very weak at the allozyme level.

Discriminant analysis showed a complex variation pattern between peatland and upland habitats at different sites. Habitats were more differentiated at sites 2 and 3 than at site 1. However, the differentiation was weak as all discriminant functions were not significant. If environmental differences between peatland and upland habitats resulted in differential selection, it would be expected that populations of the same habitat type would group together in discriminant analysis. This was not the case in my study.

Conifers generally show low levels of allozyme variation among populations. The greatest proportion of genetic diversity (often greater than 90%) is usually found within individual populations (Merkle and Adams 1987). Likely, long life span, large continuous population, wind pollination, wind dipersed seeds, outcrossing mating system, and lack of effective barriers to gene flow among populations have contributed to the lack of genetic differentiation among populations of conifers (Guries and Ledig 1981, Yeh 1981, Cheliak et al. 1988). All these factors may be important in black spruce.

2.5. Conclusion

The populations of black spruce had comparable levels of genetic variability with those reported for conifers. The populations or habitats were not well differentiated with the major proportion of genetic variation being within populations. Overall, there was greater differentiation among sites (related to geographic distance) than between the habitat types. There was greater differentiation among upland than among peatland populations. Populations from the two habitat types showed greater differentiation at sites 2 and 3, but were very similar at site 1.

Table 2-6. Summary of electrophoretic studies of genetic variation in conifers. N1=number of populations, N2=number of loci, P=percent of polymorphic loci (frequency of most common allele≤ 0.99), A=alleles per locus, H1=observed heterozygosity, H2=expected heterozygosity, D=genetic distance (means are weighted by the number of loci), F_{st}, and G_{st}

Species	N1	N2	P	A	H1	H2	F_{st}	G_{st}	D	Source
Abies balsamea	1	14	64.0	1.86		.150				Neale 1978
Larix laricina	36	16	50.0	1.8	.22	.22		.05	.032	Cheliak et al. 1988
L. occidentalis	19	23	30.4	1.5	.061	.082				Fins and Seeb 1986
Picea abies	70	22		2.60		.115		.052		Lagercrantz and Ryman 1990
P. abies	4	12	91.7	3.54		.341				Lundkvist 1979
P. glauca	2	18					.174		.005	Yeh and Arnott 1986
P. mariana	21	23	37.8	1.44	.120	.107	.059		.014	Yeh et al. 1986
P. mariana	6	14	20.4	2.31	.222	.229	.010		.0031	Boyle and Morgenstern 1987
P. mariana (peatland)	1	13		3.00	.240	.242		.009	.0051	Boyle et al. 1990
P. mariana (upland)	1	13		2.77	.202	.240				Boyle et al. 1990
P. mariana (peatland)	5	15	57.3		.23		.048		.016	O'Reilly et al. 1985
P. mariana (upland)	5	15	47.5		.21		.069		.027	O'Reilly et al. 1985
P. sitchensis	3	18				.199			.014	Yeh and Arnott 1986
P. sitchensis	10	24	51.0	2.22		.154		.079	.014	Yeh and El-Kassaby 1980
Pinus attenuata	4	32	55.0	1.7		.131			.03	Millar et al. 1988
P. banksiana	3	21		2.1	.115		.024		.005	Dancik and Yeh 1983
P. brutia brutia	10	30	43.0	1.53		.118				Conkle et al. 1988
P. brutia eldaric	1	30	37.0	1.37		.075				Conkle et al. 1988
P. brutia pithyus	1	30	30.0	1.30		.097				Conkle et al. 1988
P. brutia stankewiczii	2	30	40.0	1.43		.118				Conkle et al. 1988
P. contorta bolanderi	1	42	58.0	1.62		.109				Wheeler and Guries 1982
P. contorta contorta	5	42	65.0	1.81		.126			.0066	Wheeler and Guries 1982
P. contorta latifolia	24	42	69.0	1.86		.118			.0023	Wheeler and Guries 1982
P. contorta latifolia	5	21		2.5	.184		.018		.005	Dancik and Yeh 1983
P. contorta latifolia	4	6	44.0	1.33	.135	.144	.008			Knowles 1984
P. contorta latifolia	17	23	64.0		.174	.165				Yeh et al. 1985
P. contorta latifolia	9	25	56.7	1.90	.161	.160		.041		Yeh and Layton 1979

Table 2-6. continued

Species	N1	N2	P	A	Hobs	Hexp	F_{st}	G_{st}	D	Source
P. contorta murrayana	2	42	73.0	1.94		.128			.005	Wheeler and Guries 1982
P. flexilis	2	10				.32	.022			Schuster et al. 1989
P. halepensis(eastern)		30	23.0	1.23		.055				Conkle et al. 1988
P. halepensis(western)		30	23.0	1.23		.035				Conkle et al. 1988
P. halepensis	17	30		1.14		.045	.129			Grunwald et al. 1986
P. halepensis	19	30	15.0	1.10		.040			.02	Schiller et al. 1986
P. longaeva	5	14	78.6	2.35		.364				Hiebert 1977
P. jeffreyi	14	20				.222		.138	.0185	Furnier and Adams 1986
P. monticola	28	12	65.0	1.7		.18		.148	.031	Steinhoff et al. 1983
P. muricata	7	32	47.0	1.6		.118		.22	.04	Millar et al. 1988
P. muricata	18	45		1.5	.061	.077				Millar 1989
P. oocarpa	1	32	65.0	1.9		.270				Millar et al. 1988
P. ponderosa	10	23		1.7		.127			.122	O'Malley et al. 1979
P. pungens	3	15	40.0	1.33		.144				Feret 1974
P. radiata	5	32	58.0	1.9		.141		.13	.03	Millar et al. 1988
P. resinosa	5	9	0.0	1.00		.000			.0000	Fowler and Morris 1977
P. rigida	11	21		3.1	.138	.146	.024	.026	.007	Guries and Ledig 1982
P. strobus		17	52.9	2.06		.330				Eckert et al. 1981
P. torreyana	2	59		1.03		.000		1.0	.086	Ledig and Conkle 1983
Pseudotsuga menziesii	11	21	68.8	2.19		.155		.026	.0037	Yeh and O'Malley 1980
P. menziesii	4	27	54.0	1.79		.171		.068		El-Kassaby and Sziklai 1982
P. menziesii	11	21	68.0			.175				Yeh 1981
P. menziesii	22	27	71.7	2.46		.178		.001	.0013	Merkle and Adams 1987
P. menziesii	12	18	70.1	2.30	.155	.164				Moran and Adams 1986
Quetta pine	1	30	30.0	1.30		.082				Conkle et al. 1988
Thuja plicata	8	19	16.0	1.17		.040	.033			Yeh 1988
Mean		24	51.3	1.74	.150	.131				The Above studies
Mean of 20 conifers		20	67.7	2.29		.207				Hamrick et al. 1981
Picea mariana	6	28	55.9	1.85	.157	.166	.010		.0004	This study

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Chapter 3. Seed germination ecology

3.1. Introduction

Seed germination characteristics are, at least partially, under genetic control, and the pattern of behavior is likely to have resulted from selection (Whittington 1972). It is widely accepted that germination responses are strongly selected for under natural conditions; and for different populations of a species, responses to environmental factors represent adaptation to locally significant selective pressures (Thompson 1983).

Differences in seed germination among populations within species have been reported for several tree species, including Thuja occidentalis L. (Habeck 1958), Casuarina cuminghamiana and C. gluaca (El-Lakany and Shepherd 1983), Juniperus virginiana (Haverbake and Comer 1985), and Pinus brutia (Isik 1986). It also appears that there is some correlation between the environmental requirements for germination and the ecological conditions of the seed's origin (Mayer and Poljakoff-Mayber 1982). Provenance differences in seed germination response to moisture stress have been explained by the moisture conditions of the provenances in Pinus (P. monticola, Squillace and Bingham 1958, P. ponderosa, Moore and Kidd 1982, P. halepensis, Falusi et al. 1983), and Eucalyptus (E. camaldulensis, E. nitens, and E. obliqua, Gibson and Bachelard 1987). Germination response to temperature has been reported to be correlated with altitude in Betula verrucosa, B. nana, and B. pubescens (Makhnev and Makhneva 1979) and latitude in Picea glauca (Fraser 1971).

The conditions required for seed germination are an adequate supply of water, a suitable temperature and composition of gases, and, for some seeds, light. Among these, probably the most crucial factor in determining germination of seeds in the soil is a suitable combination of temperature and moisture (Mayer and Poljakoff-Mayber 1982). However, the effects of the combination have not been widely studied in seed germination of trees. Nevertheless, it has been shown that the interaction of temperature and moisture stress has significant effects on seed germination of sweetgum (Bonner and Farmer 1966), eastern cotton wood (Farmer and Bonner 1967), Scotch pine (Domingo 1976), Engelmann spruce, and lodgepole pine (Kaufmann and Eckard 1977).

In black spruce, Picea mariana (Mill.) B.S.P., Fraser (1970) reported that different provenances have different germination responses to temperatures. Morgenstern (1969) attributed significant population differences in germination rate to the soil moisture regime of the seed's origin. Fowler and Mullin (1977) found significant germination differences among sites, but not between upland and peatland habitats. However, Trueland (1980) and Adams (not dated) found germination differences between peatland and upland

black spruce seeds.

Peatland and upland are very different habitats. In western Canada, the soil is cooler and wetter in peatlands (Van Cleve et al. 1981). These differences between the two habitats might result in differential selection pressures on seed germination responses of plant species, such as black spruce, which grow on both of them. Therefore, it was hypothesized that black spruce seeds from upland trees would germinate better at high temperatures and/or low water potentials than seeds from peatland trees.

3.2. Materials and Methods

Seeds used in this study were from the same trees of the 3 pairs of peatland and upland populations studied in Chapter 2: Allozyme variation. The seeds were not dormant. The experimental conditions (factors) controlled were temperatures (15, 25, and 35°C) and water potentials (0, -0.5, -1.0, and -1.5 MPa) combined in full factorial. Three germinators were used to set the 3 temperatures, resulting in a simple split-plot experimental design with temperatures as whole plots. The temperatures were checked daily, using thermometers placed in the germinators, and controlled precisely. Polyethylene glycol 6000 (PEG) (Fair Lawn, New Jersey, USA) was added to distilled water to provide the appropriate water potentials at each temperature. The concentrations of PEG were calculated by extrapolation of the equation given by Michel and Kaufmann (1973), and the water potentials produced were checked with a Wescor Dew Point Microvoltmeter HR-33T. The solutions and the control contained 0.025% Thiram to prevent fungus infection.

At least, 35 trees per population had enough seeds for the planned experiments. Three seeds per tree from 35 trees in each population were bulked to form one replicate, and 4 replicates were used for each treatment combination. The 105 seeds of each replicate were sown on a filter paper (# 3, Whatman, England) in a petri dish (diameter 9cm). Five ml of the solution with appropriate water potential was added to each petri dish, and the petri dish was wrapped with Saran Wrap to prevent water evaporation.

Seeds were germinated in the dark and only exposed to light when germinants were being counted. Germinants (radicle breaking seed coat) were counted and removed once a day for 30 days. Then, a cutting test was done on the remaining seeds to obtain the total number of filled seeds for each replicate. Total germination percent (G%=number of germinants/number of filled seeds) and the days to reach 50% of the G% (R50) were calculated for each replicate. ANOVA was conducted using SPSS-PC (Norusis 1988). When significant factors or lower order interactions were involved in significant higher order interactions, SNK-test was only carried out for the means of the highest order significant interaction. Significance level ANOVA and SNK-test was $p \leq 0.05$.

3.3. Results

At water potential lower than -0.5 MPa, no germination occurred. At 15°C and 0 MPa or -0.5 MPa, or 35°C and -0.5 MPa, only a few seeds germinated sporadically; thus, R50 was not calculated for these treatments. Therefore, the data were analysed by 3 ANOVA's, and these ANOVA results are summarized in Table 3-1.

TABLE 3-1. ANOVA results for total germination percent (G%), and R50 (number of days to 50% of the G%)

Source	G%[1]		R50			
	df	P-value	df	P-value[2]	df	P-value[3]
T (temperature)	2	0.000			1	0.002
W (water potential)	1	0.000	1	0.000		
S (site)	2	0.722	2	0.004	2	0.000
H (habitat)	1	0.037	1	0.000	1	0.000
T x W	2	0.000				
T x S	4	0.625			2	0.458
T x H	2	0.000			1	0.375
W x S	2	0.312	2	0.418		
W x H	1	0.512	1	0.798		
S x H	2	0.768	2	0.000	2	0.000
T x W x S	4	0.393				
T x W x H	2	0.291				
T x S x H	4	0.065			2	0.853
W x S x H	2	0.466	2	0.041		
T x W x S x H	4	0.003				

[1] G% by S, H, T (15, 25, 35°C), W (0, -0.5 MPa), and their interactions.
[2] R50 by S, H, W (0 and -0.5 MPa), and their interactions at temperature 25°C.
[3] R50 by S, H, T (25, 35°C), and their interactions at water potential 0 MPa.

The 4-way interaction (TEMPERATURE x WATER POTENTIAL x SITE x HABITAT) was significant on G% (Table 3-1), thus, its means were examined further by SNK-test (Table 3-2). At 0 MPa, G% was much lower at 15°C than at 25 or 35°C. At -0.5 MPa, seeds germinated much better at 25°C than at 15 or 35°C. On average, G% decreased from 65.75% at 0 MPa to 28.91% at -0.5 MPa. At 15°C (0 or -0.5 MPa), seeds from upland populations had a higher G% than those from peatland although the difference between habitats was only significant for site 1 at 0 MPa. At -0.5 MPa and 35°C, site 1 had a higher G% in the upland population than in the

peatland. However, the opposite occurred between habitats in site 3 under the same conditions.

TABLE 3-2. Means of total germination percent by WATER POTENTIAL by TEMPERATURE by SITE by HABITAT

		WATER POTENTIAL (MPa)					
		0			-0.5		
		TEMPERATURE			TEMPERATURE (°C)		
SITE	HABITAT	15	25	35	15	25	35
1	Peatland	11.02a	91.94a	91.38a	7.11a	66.05a	6.62a[1/]
	Upland	23.43b	91.86a	85.99a	12.54a	58.05a	18.83b
2	Peatland	13.95ab	92.97a	86.62a	4.46a	67.38a	17.21b
	Upland	21.11b	93.35a	83.81a	10.64a	65.88a	16.67b
3	Peatland	13.50ab	91.91a	87.49a	5.49a	61.56a	18.60b
	Upland	23.69b	92.08a	87.44a	11.20a	65.79a	6.37a
Mean	Peatland	12.85	92.27	88.50	5.69	65.00	14.14
	Upland	22.74	92.43	85.75	11.46	63.24	13.96
Overall mean		17.78	92.35	87.12	8.57	64.12	14.05

[1/] In each column means marked by different letters are different at 5% significance level by SNK-test.

In ANOVA testing effects of HABITAT (H), WATER POTENTIAL (W) (0, -0.5 MPa), and SITE (S) on R50 at 25°C, W x S x H was significant. Thus, the means of the 3-way interaction were analysed further by SNK-test (Table 3-3).

TABLE 3-3. Means of R50 (number of days to 50% of the total germination percent) by WATER POTENTIAL by SITE by HABITAT at temperature 25°C

	WATER POTENTIAL (MPa)					
	0			-0.5		
	SITE			SITE		
HABITAT	1	2	3	1	2	3
Peatland	3.56a	3.71a	4.37b	6.24cd	6.66c	7.40e[1/]
Upland	3.22a	3.32a	3.41a	6.65c	5.78d	6.02cd

[1/] Means marked by different letters are different at 5% significance level by SNK-test.

Generally, seeds from upland populations germinated faster than those from peatland, with the largest difference between habitats occurring for site 3 at either 0 or -0.5 MPa. Although seeds from the peatland population at site 1 germinated faster at -0.5 MPa than those from upland, the difference was not significant. The variation among R50's for upland populations was smaller than for peatland populations at 0 or -0.5 MPa. Among the peatland populations, site 3 had significantly slower germination than site 1 or 2. Overall, water potential -0.5 MPa greatly reduced germination speed. At -0.5 MPa, it took the seeds almost twice as long to reach R50.

In ANOVA testing effects of SITE (S), HABITAT (H), and TEMPERATURE (25, 35°C) on R50 at 0 MPa, effects S, H, and S x H were significant (Table 3-1). So, the means of the 2-way interaction were examined further by SNK-test (Table 3-4). Upland populations had similar R50's which were significantly smaller than those of peatland populations. Among the 3 sites, the largest difference between habitats occurred at site 3, where the peatland population showed much slower germination. Peatland populations had significantly different R50's, with the biggest difference between site 3 and sites 1 and 2.

TABLE 3-4. Means of R50 (number of days to 50% of the total germination percent) by SITE by HABITAT (averaged for 25°C and 35°C) at 0 MPa

	SITE		
HABITAT	1	2	3
Peatland	3.50b[1/]	3.69c	4.28d
Upland	3.15a	3.25a	3.26a

[1/] Means marked by different letters are different at 5% significance level by SNK-test.

3.4. Discussion

When seeds are collected from natural populations and used in studies of germination ecology, response differences among populations include both genetic and environmental components (Quinn and Colosi 1977). To study the potentially adaptive germination response as determined by environmental conditions, it is recommended that seeds from natural populations be grown under a variety of uniform environments for one generation before studies are initiated. However, this is very difficult, if not impossible, to do for some trees which have a long vegetative growth period. Thus, to

do the study for trees, the least we should do is to examine the interaction effects between populations from different environments and test conditions on germination response.

If germination responses have adaptive significance, local populations of a widely distributed species would show adaptive variability in such responses (Quinn and Colosi 1977). It was hypothesized that seeds from peatland and upland habitats would differ in their seed germination response as a result of adaptive evolution. Thus, they would germinate better in conditions similar to those of their native habitats. To support this hypothesis, interactions between HABITAT and TEMPERATURE (T), or WATER POTENTIAL (W), or T x W should be significant with seeds from upland trees germinating better at high temperature and/or low water potential. In this study, W x T x H, or W x H did not affect G%. Although there was a significant T x H effect on G%, this was due to the better germination by upland populations at low temperature. At low water potential (-0.5 MPa) and high temperature (35°C), G% difference between habitats varied with sites. The effect of T x H was not significant on R50, and the effect of W x H varied with sites. Thus, the interactions to test the hypothesis did not conform to the expectations. However, the analyses demonstrated significant site-dependent effects. These might reflect the influence of local site-specific selection.

Several explanations are possible for the lack of concordance of results with expectation. First, selection for different genotypes is expected to be severe in the year of germination and establishment of seedlings when moisture and temperature are most critical. However, the success of germination and establishment may also be determined by the availability of 'safe sites' (Harper 1977), which may differ little between habitats with respect to selection pressure.

While it was hypothesized that adaptation of upland black spruce trees would result in better germination at lower moisture and higher temperature conditions, drought or heat resistant genotypes could be selected for in peatlands during the dry season as the upper peat surface may be very dry and warm (Denyer and Riley 1964, R. Rothwell, unpublished).

Thirdly, the difference in germination environments between upland and peatland may be less than originally expected for the following reasons: 1. Since both peatland and upland black spruce are fire-origin, the seed bed conditions may be similar between the 2 habitats; and 2. Germination is likely to occur during the moist period (early spring) when there are no moisture differences between the habitats.

Forthly, even if drought or heat resistant genotypes are selected on dry upland sites, populations must be sufficiently isolated to accumulate gene differences (Ford 1964). Black spruce is an outcrossing species, and gene

exchange among adjacent populations should be present. If gene exchange overcomes selection pressures, little or no habitat differences in germination response would appear.

Finally, the experimental design may not have been appropriate to reveal habitat differentiation or the differentiation may have been too weak to be detected.

Seeds from upland populations germinated more at 15°C, and generally they germinated faster than those from peatland. Habeck (1958) reported in white cedar that seeds from upland stands had higher germination than those from lowland. Morgenstern (1969) found that germination speed and percent decreased with increasing soil moisture regime of the seed's origin in black spruce. Preconditioning of seed by climate and soil affects subsequent germination behavior (Rowe 1964). Differences in seed composition and germination exist between seeds from trees grown on fertile and infertile soils (Youngberg 1952). Soil moisture and fertility of the seed origin influence seed development and seed vigor (Delouche 1980). Vigorous seeds germinate promptly and produce rapidly developing seedlings under favorable and unfavorable environmental conditions (Maguire 1977). The lower nutrient availability and stressful moisture conditions of peatland may be reflected in low seed vigor and consequently poor seed germination of peatland trees. Therefore, the germination differences between habitats might reflect the influence of the maternal environment rather than genetic effects.

The variation in R50 was smaller among upland than among peatland populations, which might indicate that the environmental conditions for seed development are more similar among upland than among peatland populations. This contrasts with the isozyme results of O'Reilly et al. (1985) who found greater variation among upland than among peatland populations in the same species. Within the peatland habitat, site 3 was the most divergent. This might indicate that the environmental differences and consequentially the resulting population differences in seed germination were the greatest between the peatland at site 3 and at sites 1 and 2.

Low water potential reduced total germination and germination speed. At less than -0.5 MPa, no germination occurred. Increased water stress has been shown to reduce total germination and slow down germination speed in other species (Bewley and Black 1982). The effects of water stress on germination are attributed to the reduced rate of seed hydration under low water potential (Therios 1982).

Temperature had a major influence on seed germination (Table 3-2). It is interesting to compare the results with those of Fraser (1970). Black spruce seeds from 6 provenances in his study showed germination from 62 to 99% at 15.6°C but only 5 to 40% at 35°C. In this study, under 0 MPa germination was 11 to 23% at 15°C and 84 to 91% at 35°C.

Thus, seeds in this study germinated better at higher temperature and worse at lower temperature than those in his study. This difference in germination response to temperature was unexpected. Five of his seed provenances were from more southerly (45°20' to 50°07'N) and easterly (77°26' to 91°54'W) locations than those in my study (53°25' to 55°08'N; 114°15' to 116°01'W).

Temperature alone can be an important ecological factor in seed germination. It has been reported that the optimum temperature for seed germination is correlated with altitude in Betula species (Makhnev and Makhneva 1979) and with latitudes in Picea gluaca (Fraser 1971). Many species show adaptations in their germination requirement in accordance with temperature conditions prevailing in different areas (Mayer and Poljakoff 1982). Following this, the opposite variation pattern would be expected as the temperatures of seed origins in my study may be lower than those in Fraser (1971). The reason for this unexpected difference might be seed preconditioning by temperature (Rowe 1964). This implies that seeds in his study developed and matured in cooler environments than those in this study. However, this cannot be verified as the exact location and year of collection of seeds in his study are unknown.

3.5. Conclusion

Seeds from upland and peatland did not show expected adaptive germination responses to temperature, moisture, or their interaction. But, differentiation among the 6 populations was significant. Overall, seeds germinated best at 25°C, and seeds from peatland populations germinated more slowly and poorly at low temperature. Differences in germination among populations or between habitats were most likely due to maternal effects and/or locally site-specific selection.

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Chapter 4. Morphology, physiology, and plasticity

4.1. Introduction

Variation among individuals within a species is universal, and no two individuals are genetically identical in natural populations (Merrell 1981) except individuals produced asexually from the same genotypes. This provides the raw material for natural selection to work on. Natural selection is selection by environment. It is the mechanism of adaptation. Natural selection operates on phenotypes directly and on genotypes indirectly. It acts to eliminate the less adapted ones, thus producing populations better adapted to relatively stable environmental conditions or able to adapt to changing environmental conditions (Merrell 1981). Therefore, populations of a species exposed to different selection pressures are expected to differ in morphology, physiology, and response to environment (plasticity).

Morphological and physiological traits have been widely used to study population differentiation in forest trees. They reveal the adaptive strategies of the populations when the interaction between populations and their environments are examined. It has been shown that variation detected by these quantitative traits is greater than that revealed by allozymes (e.g., Cheliak et al., 1988, Farmer et al. 1988, Loopstra and Adams 1989, Moran and Adams 1989), and that variability in one character set is not a good predictor of that in another (Linhart et al. 1989). Moreover, different traits subject to selection at different life stages (e.g., germination at early and reproduction at late stages) and the same traits are subject to selection to a different extent depending on life stages (e.g., survival may be more subject to selection at early than at late stages). Therefore, although black spruce populations from peatland and upland habitats did not show ecotypic differentiation in allozymes (Chapter 2) and in seed germination (Chapter 3), it was unknown if they were differentiated in morphological and physiological traits.

Variation among geographically distant populations of trees has been observed to be almost universal on the basis of morphological traits (Squillace and Bingham 1958). Differentiation in these traits has also been detected among geographically adjacent populations (Squillace and Bingham 1958, Hermann and Lavender 1968, Musselman et al. 1975, Rehfeldt 1988) and edaphic populations (Kruckeberg 1967, Teich and Holst 1974, Jenkinson 1977). Information from these studies on survival, growth, cold hardiness, and disease and pest resistances has permitted tree breeders to identify suitable seed sources for use in reforestation.

In a particular environment, a high photosynthetic efficiency and a low respiration activity are of great importance in determining the success of a given plant

(Bjorkman 1966). Although there is considerable intraspecific and interspecific genetic variation in photosynthesis (Luukkanen 1972), there has been relatively little research on genetic variation of photosynthetic traits in trees (Isebrands et al. 1988). Nevertheless, it has been shown that adjacent altitudinal populations differed with respect to their temperature optimum for photosynthetic CO_2-uptake in Abies balsamea (L.) Mill. (Fryer and Ledig 1972) and in Eucalyptus pauciflora sieb. ex spreng. (Slatyer et al. 1978). Significant localized differences among altitudinal Acer saccharum Marsh. populations were detected in photosynthesis and respiration (Ledig and Korbobo 1983). Pinus ponderosa Laws. populations from two genetic clines (coastal and interior) have been found to differ in photosynthesis, stomatal conductance, and water use efficiency (Monson and Grant 1989).

Phenotypic plasticity is a widespread phenomenon in higher plants. It is under genetic control, and therefore subject to natural selection (Macdonald and Chinnappa 1989). Population differentiation has been detected for the mean (genotypic) value, amount of plasticity, and pattern of plasticity of traits, and these characters were independent of each other (Schichting and Levin 1986, Macdonald and Chinnappa 1989). Phenotypic plasticity has been used as a trait to study population differentiation for many plant species (Wilken 1977, Marshall et al. 1986, Schichting and Levin 1986, 1990, Taylor and Aarssen 1988, Macdonald and Chinnappa 1989), but not yet in forest trees. To understand genetic variation within and among populations more completely, plasticity should be included as an independent trait just as allozymes or other phenotypic traits.

In studies of black spruce population differentiation, a physiological approach has not yet been used. Based on morphological traits, a clinal variation pattern has been found among black spruce populations in Ontario (Morgenstern 1969a, b) and in the Maritimes (Fowler and Park 1982). In Newfoundland, variation in morphological traits was ecotypic with respect to regional and altitudinal climates (Khalil 1975). However, Hall (1986) found that the variation in height growth in Newfoundland populations was both clinal and ecotypic.

Differentiation for morphological, survival, and physiological traits has been demonstrated between upland and lowland white cedar (Habeck 1958, Musselman et al. 1975). Although black spruce is analogous to white cedar in its site preference (Parker et al. 1983), its ecotypic differentiation between peatland and upland habitats has not been conclusively demonstrated. Fowler and Mullin (1977) studied 15 upland and 15 peatland black spruce populations from 3 locations in northern Ontario. Their results showed significant differences (seed size, germination, and seedling survival and height growth) among locations, but

not between habitats. Parker et al. (1983) measured 17 morphological traits (cone, needle, and twig) from trees of 3 black spruce stands (well drained upland to very wet peatland) in northwestern Ontario. They found very little variation among sites. On the other hand, variation between upland and peatland black spruce in Ontario has been detected for seed weight and germination (Trueland 1980), root growth (Fung 1975, Trueland 1980, Lee 1984), and germination and wilting responses to moisture stress (Adams not dated).

The final portion of my research was to study population differentiation of peatland and upland black spruce in Alberta using morphological and physiological traits, and their plasticities. It consisted of 2 experiments. Experiment 1 was to test if seedlings from upland sites would perform better under conditions of limited moisture and light than those from peatland sites. Experiment 2 was to test if seedlings from peatland sites would perform better in cold and nutrient poor soil than those from upland sites.

4.2. Materials and methods

Seeds used in this study were from the same trees of the 3 pairs of peatland and upland populations studied in Chapter 1 (Table 2-1). Individual seed weight ranged from 0.7 to 1.8 mg. In this study, however, only those seeds in the range of 1.1 to 1.4 mg were used.

Experiment 1

Seedlings were grown in (14cm x 2.5cm x 3cm = 105 ml) Spencer-lemaire containers filled with commercial peatmoss. Each container tray had 65 cells. Seeds were cold-stratified for 2 weeks at 3-5°C and then germinated at 25°C in a germinator at 16 hour photoperiod for 5 days. Germinants with radicle about the length of the seed were planted.

In this experiment, factors were LIGHT (100% or 20% of the full light in the greenhouse) and MOISTURE (wet - soaking once/day; medium - soaking once/5 days; and dry - soaking once/10 days in fertilizer solution of 0.5 g 20-20-20 NPK fertilizer per litre of tap water). In a full factorial design, these yielded 6 treatments. With a light meter, it was found that 3 layers of shade cloth reduced light to 20% of full light. Thus, 20% of the full light condition was created by placing seedlings inside wooden frames covered with 3 layers of the shade cloth.

Two seedlings from each of 18 trees per population were grown in each of the 6 environments (a total of 1296 seedlings). To simplify harvesting, the experiment was broken into 6 consecutive planting and harvesting days. On each planting day, 2 germinants from each of 3 trees per population were planted into each of 6 different container trays which were randomly assigned to one of the 6

treatments. The seedlings from 3 trees per population which were planted on the same day were then harvested on the same day, 120 days later.

Treatments were started 2 weeks after planting. In the first 2 weeks, seedlings were placed in 100% light and watered daily. Growing conditions were controlled at a 16 hours photoperiod and, depending on the conditions outside, temperatures varied from 20 to 30°C inside the greenhouse.

At the end of the growing period, measurements were taken and seedlings were harvested on 6 consecutive days. The harvesting day was the day when the next soaking was due but had not been applied.

Physiological variables, net assimilation, stomatal and mesophyll conductance to CO_2, and water use efficiency, were measured for 2 seedlings from each of 1 or 2 trees per population were measured on each harvesting day for each treatment. Physiological measures were made on 120 seedlings on each harvesting day. A total of 720 (2 seedlings x 10 trees x 6 populations x 6 treatments) seedlings were measured for physiological variables.

Methods and equipment for physiological measurements were similar to those of Macdonald and Lieffers (1990). Gas exchange was measured in an open system using a portable infrared gas analyzer (IRGA, LCA-2), air pump, and cuvette (PLC-C) (Analytical Development Corporation., Hoddeson, England). When measuring, seedlings were taken into a lab, ambient air was taken from outside, and the air flow through the cuvette was 8 mL s^{-1}. A slide projector was positioned to provide 1000 uE m^{-2} s^{-1} photosynthetically active radiation (PAR) to seedlings in full light treatment; or covered with shade cloth and positioned to provide 200 uE m^{-2} s^{-1} PAR (20% of the full light, 1000 uE m^{-2} s^{-1}) to the seedlings in low light treatments. Ambient CO_2 concentration (C_a), time of measurement (T), and cuvette temperature (T_a) were also recorded.

Values for physiological variables, net assimilation (NA, μmole $CO_2 g^{-1} h^{-1}$), stomatal and mesophyll conductance to CO_2 (g_c and g_m, mmole $CO_2 g^{-1} h^{-1}$) on the basis of needle dry weight, and water use efficiency (WUE, umole CO_2/mmole H_2O) were determined using standard calculations (Caemmerer and Farquhar 1981). Leaf temperature was assumed to be the same as air temperature in the cuvette. Also, boundary layer resistance was ignored as in Macdonald and Lieffers (1990).

All seedlings (1296 - 11 (dead) = 1285) were measured for morphological variables (height (Ht), top dry weight (Dwt), and root dry weight (Dwr)). Dry weight was determined at 0.0001 g accuracy after the materials were oven-dried at 70°C for 3 days. From these, total dry weight (Tdw) and Dwt/Dwr ratio (R) were calculated for each seedling.

In data analyses, a significance level of $P \leq 0.05$ was used. Methods of analysis were ANOVA, LSD-test, and discriminant analysis. For physiological variables, stepwise regression

analysis was done first using NA, g_c, g_m and WUE as dependent and C_a, T, T_a, C_a x T, C_a x T_a, T x T_a, C_a^2, T^2, and T_a^2 as independent variables. The significant independent variables were used as covariates in ANOVA for the corresponding dependent variables. In discriminant analyses of physiological variables, residuals from 4 separate regression analyses (NA, g_c, g_m, and WUE including appropriate covariates) were used. ANOVA and discriminant analysis were done using SAS (SAS Institute Inc. 1985) and SPSS-PC (Norusis 1988) respectively.

Because there was about 0.1% seedling mortality and a few seedlings had no measurable NA, the data for morphological and physiological variables were slightly unbalanced. Thus, the type III sum of squares from SAS was used in ANOVA. SITE (S), HABITAT (H), MOISTURE (M), LIGHT (L), and HARVESTING DAYS (D) were used as fixed factors, and TREES WITHIN D, S, and H was used as a random factor in ANOVA. If a factor or an interaction was significant but also involved in a significant higher-order interaction in ANOVA, only the means of the highest-order significant interaction were examined further by LSD-test.

Experiment 2

In this experiment, factors controlled were SUBSTRATE TEMPERATURE (ambient (20°C) or low (8°C) at 5 cm from the surface) and FERTILIZATION (watering seedlings with tap water or fertilizer solution of 0.5 g 20-20-20 NPK fertilizer per litre of tap water). These resulted in 4 treatments (2 x 2).

Plastic tubes (PVC) (14.5 cm height, 4.3 cm diameter) placed in 2 plastic lined wooden boxes (200cm x 100cm x 20cm) were used to grow seedlings. One seedling was grown in each tube. The tubes and all other space were filled with commercial peatmoss. Substrate temperature was lowered in one of the boxes by circulating chilled ethylene glycol through a network of copper tubing at the bottom of the box. Each box was divided in half by an insulated divider; one side received tap water, and another, the fertilizer solution.

Other methods were similar to experiment 1, but seeds from 20 trees per population were used and there were 4 consecutive planting and harvesting days. Thus, 2 germinants from each of 5 trees per population were planted into each treatment on a given planting day. Treatments were started immediately after planting, and the growing period was 130 days. Seedlings were watered with water or fertilizer solution when the peat surface was dry. On each harvesting day, 2 seedlings from each of 2 trees per population were used for physiological measurements. A total of 384 (2 seedlings x 8 trees x 6 populations x 4 treatments) seedlings were measured for physiological variables. Physiological measurements were taken without any

disturbance to the growing medium in the boxes by cutting the top at the peat surface. All seedlings (960 - 10 (dead) = 950) were measured for morphological variables as in experiment 1. The data analysis procedures were as those described in experiment 1. In ANOVA, SUBSTRATE TEMPERATURE and FERTILIZATION were treated as split factors.

Genetic and plastic components of variance

Further analyses of the data were done in order to examine plastic and genetic components of variance for these populations in both experiments. Genetic variation within population was determined using family means (across treatments). Coefficients of variation (CV) of the family means were then calculated for each population on every trait. These gave an estimate of genetic variation within each population: CV values of each trait by SITE x HABITAT.

The amount of phenotypic plasticity of each trait was also determined. Within treatment means (across families) for each population were calculated. The CV of these means (across treatments) was then defined as the amount of phenotypic plasticity of each trait for a given population (Schlichting 1986).

The genetic variation among populations within habitats was determined as follows for each trait. Population means (across families and treatments) were calculated for every trait. The CV's of 3 population means within each habitat were calculated for every trait and used as the genetic variation among population.

For the data on genetic and plastic variation within populations, ANOVA was used to test the differences in mean population genetic variation or phenotypic plasticity among the 3 sites or between the peatland and upland habitats for each trait. Discriminant analyses were used, in a multivariate approach, to test if the 3 sites or 2 habitats differed in genetic or plasticity components of variance.

4.3. Results

Experiment 1

All traits were significantly affected by MOISTURE, LIGHT, and their interaction (Table 4-1). Peatland and upland habitats differed in height (Ht) and stomatal conductance to CO_2 (g_c), and they showed different Ht responses to moisture regimes (Table 4-1). Habitat did not differ in response to LIGHT and did not vary with sites (Table 4-1). There were differences among sites for all morphological traits, and sites differed in their morphological responses (except R, top dry weight (Dwt)/root dry weight (Dwr)) to LIGHT, to MOISTURE or MOISTURE x LIGHT (except Ht) (Table 4-1).

Means of each trait by MOISTURE x LIGHT are summarized in Table 4-2. Watering every 5 days resulted in the largest seedlings and shoot/root ratio, but watering every day produced the largest NA, g_c, and g_m (mesophyll conductance

to CO_2). As moisture decreased, NA, g_c, g_m, and WUE decreased. Compared to full light, low light reduced seedling growth, NA, g_c, g_m, and WUE, but increased R. The effects of moisture on seedling growth and WUE were more pronounced at full light, but moisture had greater effects on NA, g_c, and g_m at low light. The effects of light on Ht, Dwt, Dwr, Tdw, and WUE were most pronounced when watering was every 5 days, but on R, NA, g_c, and g_m, when watering was every 10 days. Watering every 10 days together with low light gave the smallest NA, g_c, g_m, and WUE.

TABLE 4-1. P-values of ANOVA for MOISTURE (M), LIGHT (L), HABITAT (H), SITE (S), and their interactions on: height (Ht), top dry weight (Dwt), root dry weight (Dwr), total dry weight (Tdw), Dwt/Dwr (R), net assimilation (NA), stomatal and mesophyll conductance to CO_2 (g_c and g_m), and water use efficiency (WUE) (from experiment 1)

Source	Ht	Dwt	Dwr	Tdw	R	NA	g_c	g_m	WUE
M	.000	.000	.000	.000	.000	.000	.000	.000	.000
L	.000	.000	.000	.000	.000	.000	.000	.000	.000
M x L	.000	.000	.000	.000	.001	.000	.000	.000	.000
H	.003	.095	.095	.087	.768	.164	.046	.130	.641
H x M	.040	.273	.696	.338	.905	.509	.154	.436	.285
H x L	.077	.169	.116	.147	.644	.450	.349	.534	.393
H x M x L	.057	.270	.696	.335	.212	.387	.670	.362	.178
S	.000	.000	.000	.000	.011	.659	.979	.678	.969
S x H	.264	.391	.627	.468	.132	.794	.910	.847	.707
S x M	.176	.007	.003	.005	.513	.683	.892	.509	.907
S x L	.019	.000	.000	.000	.158	.719	.841	.717	.817
S x H x M	.808	.769	.899	.816	.260	.671	.530	.600	.112
S x H x L	.995	.433	.598	.496	.104	.691	.971	.782	.092
S x M x L	.972	.013	.003	.008	.218	.774	.306	.711	.157
S x H x M x L	.751	.771	.892	.815	.469	.522	.608	.308	.469

Upland seedlings were taller and had smaller g_c than peatland ones, and the height difference between habitats was the largest when watering every day (Table 4-3).

Generally, site 3 had the smallest seedlings, but the largest R (Table 4-4). Site differences in dry weights were only significant at full light (Table 4-4). At site 3, dry weight traits were less affected by moisture and by the interaction of moisture and light (Table 4-4).

Table 4-5 summarizes the results of discriminant analyses. No discriminant function significantly separated the 6 populations, 3 sites, or 2 habitats based on morphological or physiological traits.

TABLE 4-2. Mean height (Ht), top dry weight (Dwt), root dry weight (Dwr), total dry weight (Tdw), Dwt/Dwr (R), net assimilation (NA, $\mu moleg^{-1}h^{-1}$), stomatal and mesophyll conductances to CO_2 (g_c and g_m, $mmoleg^{-1}h^{-1}$), and water use efficiency (WUE, $\mu mole\ CO_2/mmole\ H_2O$) by MOISTURE by LIGHT

Variable	LIGHT (% of full light)	MOISTURE[1/] wet	medium	dry	mean
Ht (cm)	20	5.70a	7.49b	6.11a[2/]	6.43
	100	8.31c	21.65e	16.69d	15.56
	Mean	7.01	14.57	11.40	
Dwt (g)	20	0.03a	0.04a	0.03a	0.04
	100	0.26b	1.38c	0.93d	0.87
	Mean	0.14	0.71	0.48	
Dwr (g)	20	0.01a	0.01a	0.004a	0.01
	100	0.06b	0.26c	0.22d	0.18
	Mean	0.03	0.13	0.11	
Tdw (g)	20	0.04a	0.05a	0.03a	0.04
	100	0.32b	1.64c	1.15d	1.05
	Mean	0.18	0.84	0.59	
R	20	7.97c	9.58e	8.74d	8.76
	100	4.32a	5.95b	4.38a	4.89
	Mean	6.19	7.76	6.56	
NA	20	85.06d	52.78b	26.27a	54.70
	100	89.19d	81.18cd	73.81c	81.39
	Mean	87.13	66.98	50.04	
g_c	20	1.31c	0.86b	0.67a	0.95
	100	2.07f	1.93e	1.74d	1.91
	Mean	1.69	1.40	1.21	
g_m	20	.346d	.213b	.100a	.220
	100	.351d	.322d	.288c	.320
	Mean	.348	.267	.194	
WUE	20	0.80ab	0.78ab	0.73a	0.77
	100	1.26c	1.35c	0.87b	1.16
	Mean	1.03	1.07	0.80	

[1/] Wet, medium, and dry correspond to soaking seedlings once every day, every 5, or 10 days respectively.
[2/] For each trait, means marked by different letters are significantly different at 5% level by LSD-test.

TABLE 4-3. Mean height (Ht, cm) by MOISTURE x HABITAT, and mean stomatal conductance to CO_2 (g_c, mmole $CO_2 g^{-1} h^{-1}$) by HABITAT

Variable	Habitat	Moisture[1/] wet	medium	dry	mean
Ht	Peatland	6.47a	14.30e	11.33c[2/]	10.73
	Upland	7.53b	14.84d	11.47c	11.30
g_c	Peatland				1.50
	Upland				1.36

[1/] As for Table 2.
[2/] The means of the interaction marked by different letters are significantly different at 0.05% level by LSD-test.

TABLE 4-4. Mean height (Ht, cm) by SITE x LIGHT averaged for all 3 moisture regimes, mean top dry weight (Dwt, g), root dry weight (Dwr, g), and total dry weight (Tdw, g) SITE x MOISTURE x LIGHT, and mean Dwt/Dwr (R) by SITE[1/]

		MOISTURE								
		Wet		medium		dry[2/]		Mean of all MOISTURE		
		LIGHT								
	SITE	20%	100%	20%	100%	20%	100%	20%	100%	mean
Ht	1							6.8a	16.6e	11.6
	2							6.6ab	15.9d	11.2
	3							6.0b	14.5c	10.3
Dwt	1	.03d	.29a	.05d	1.52a	.03d	1.01a			.49
	2	.03d	.27ab	.04d	1.43b	.03d	.93b			.46
	3	.03d	.21b	.04d	1.19c	.03d	.85c			.39
Dwr	1	.01d	.07a	.01d	.30a	.01d	.23a			.10
	2	.01d	.07a	.01d	.26b	.004d	.22a			.09
	3	.01d	.05b	.004d	.21c	.003d	.20b			.08
Tdw	1	.04d	.36a	.06d	1.83a	.04d	1.24a			.60
	2	.04d	.34ab	.05d	1.69b	.03d	1.16a			.55
	3	.04d	.26b	.04d	1.40c	.03d	1.05b			.49
R	1									6.62a
	2									6.84ab
	3									7.07b

[1/] For individual variables, the means marked by different letters are significantly different at 0.05% level by LSD-test.

[2/] As for Table 2.

TABLE 4-5. Results of discriminant analysis for the data variables of morphology and physiology

Source	Discriminant function	% of variance	Canonical r	p value	Mean % of grouped cases correctly classified
Morphological data					
6 populations	1	63.56	.1223	.0643	20.00
	2	27.72	.0811	.5207	
	3	5.94	.0376	.8501	
	4	2.78	.0258	.6540	
2 habitats	1	100.00	.0773	.1045	53.70
3 sites	1	97.97	.1062	.0629	36.34
	2	2.03	.0154	.9596	
Physiological data					
6 populations	1	67.45	.1561	.8373	19.79
	2	26.25	.0981	.9716	
	3	4.82	.0422	.9897	
	4	1.48	.0234	.9013	
2 habitats	1	100.00	.0591	.8560	52.08
3 sites	1	97.46	.1243	.6404	37.50
	2	2.54	.0202	.9845	

The discriminant plots (Fig. 4-1, 4-2) show that site 3 was the most different among the sites, and the difference between habitats was the largest at site 3. Upland populations were grouped to the upper-right part of the plot based on morphological traits (Fig. 4-1) and to the lower-left part of the plot based on physiological traits (Fig. 4-2), but in both cases the separation between them and the peatland populations was not significant (Table 4-5). Differences among peatland populations were similar to those among upland based on morphological traits (Fig. 4-1). However, based on physiological traits, there were greater differences among peatland than among upland populations (Fig. 4-2).

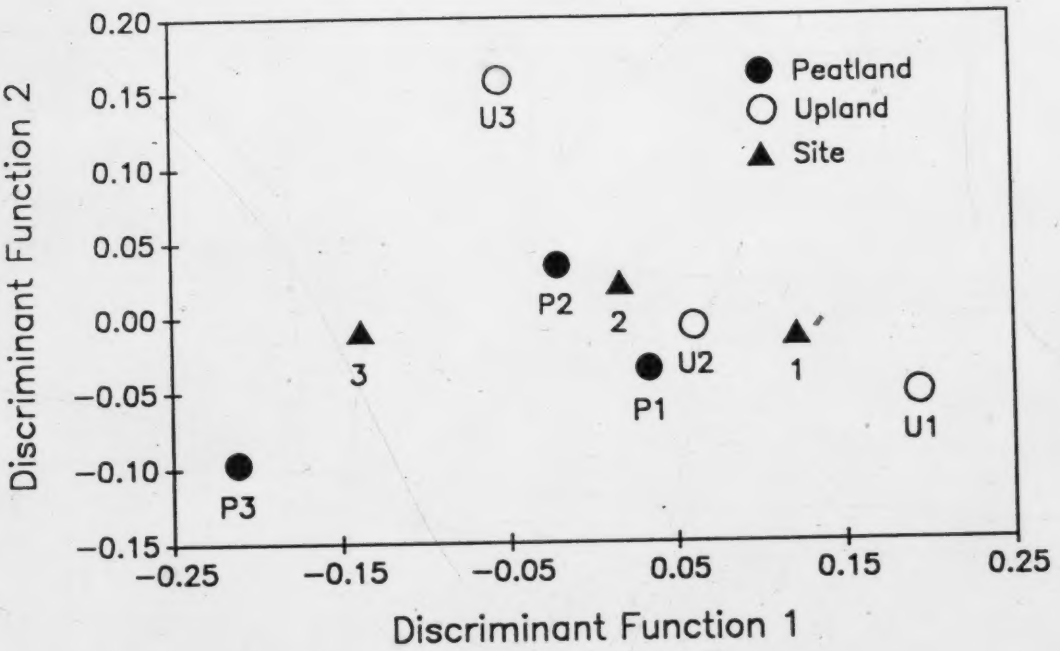

FIG. 4-1. Discriminant plot for the 6 black spruce populations at the 3 sites based on morphological data in experiment 1. Cases contained in P1, P2, P3, U1, U2, U3, and site 1, 2, and 3 were 1060 (212 seedlings x 5 variables, height, and top, root dry weight, top/root dry weight, and total dry weight), 1075 (215 x 5), 1080 (216 x 5), 1075 (215 x 5), 1075 (215 x 5), 1065 (213 x 5), 2130 (426 x 5), 2150 (430 x 5), and 2145 (429 x 5) respectively.

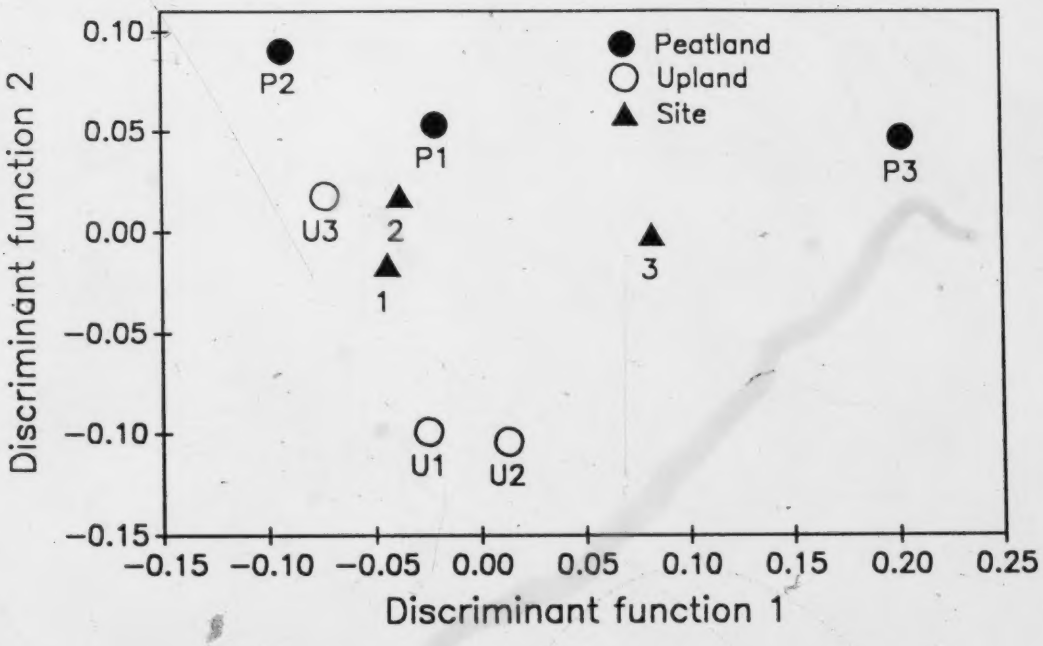

FIG. 4-2. Discriminant plot for the 6 black spruce populations at the 3 sites based on physiological data in experiment 1. Cases contained in P1, P2, P3, U1, U2, U3, and site 1, 2, and 3 were 460 (115 seedlings x 4 variables, net assimilation, stomatal and mesohyll conductances to CO_2, and water use efficiency), 456 (114 x 4), 456 (114 x 4), 476 (119 x 4), 464 (116 x 4), 936 (234 x 4), 920 (230 x 4), and 920 (230 x 4) respectively.

Experiment 2

Results of ANOVA are given in Table 4-6. All traits were significantly affected by FERTILIZATION (F), while only physiological traits and Ht were significantly affected by SUBSTRATE TEMPERATURE (C). The interaction of F x C influenced all traits significantly except Ht, R, and WUE. Peatland and upland habitats differed in dry weights but not R. The 2 habitats responded similarly to environmental conditions (C, F, and C x F). Differences between the 2 habitats in Dwt and Tdw and in the effect of fertilization on WUE were variable among the 3 sites. Site differences were significant for all morphological traits. The 3 sites showed similar response to C, but varied in the effect of fertilization on all dry weight traits and R.

TABLE 4-6. P-values of ANOVA for effects of FERTILIZATION (F), SUBSTRATE TEMPERATURE (C), SITE (S), HABITAT (H), and their interactions on: height (Ht), top dry weight (Dwt), root dry weight (Dwr), total dry weight (Tdw), dwt/dwr (R), net assimilation (NA), stomatal conductance and mesophyll conductance to CO_2 (g_c and g_m), and water use efficiency (WUE) (from experiment 2)

	Variable								
Source	Ht	Dwt	Dwr	Tdw	R	NA	g_c	g_m	WUE
F	.000	.000	.000	.000	.000	.001	.001	.000	.000
C	.000	.837	.499	.763	.192	.000	.000	.000	.001
C x F	.466	.016	.000	.006	.168	.000	.000	.000	.143
H	.771	.007	.037	.006	.750	.979	.782	.907	.430
H x C	.078	.183	.654	.215	.328	.715	.596	.789	.657
H x F	.082	.498	.256	.711	.052	.340	.376	.361	.932
H x C x F	.483	.875	.181	.704	.352	.849	.667	.793	.237
S	.000	.000	.000	.000	.003	.928	.379	.950	.134
S x H	.329	.002	.184	.003	.283	.964	.917	.960	.986
S x C	.561	.224	.154	.179	.644	.949	.809	.835	.542
S x F	.794	.012	.024	.010	.016	.938	.900	.937	.582
S x H x C	.658	.163	.557	.221	.089	.588	.863	.551	.318
S x H x F	.581	.330	.640	.511	.197	.834	.491	.530	.024
S x C x F	.244	.874	.521	.830	.435	.885	.408	.981	.706
S x H x C x F	.479	.846	.148	.698	.378	.994	.739	.963	.411

Means of all the traits by F x C are given in Table 4-7. Without fertilization, seedlings were smaller, had a smaller R, and a lower NA, g_c, g_m, and WUE. The fertilization effects were more pronounced at high substrate temperature. At low substrate temperature, seedlings were shorter, but had higher WUE. When seedlings were fertilized, low

TABLE 4-7. Mean height (Ht, cm), top dry weight (Dwt, g), root dry weight (Dwr, g), total dry weight (Tdw, g), Dwt/Dwr (R), net assimilation (NA, μmole $CO_2 g^{-1} h^{-1}$), stomatal and mesophyll conductance to CO_2 (g_c and g_m, mmole CO_2/g/hour), and water use efficiency (WUE, μmole CO_2/mmole H_2O) by FERTILIZATION and SUBSTRATE TEMPERATURE[1/]

Variable	SUBSTRATE TEMPERATURE	FERTILIZATION yes	no	mean
Ht	Low			14.16
	High			14.80
	Mean	16.74	12.22	
Dwt	Low	.91b	.44a	.68
	High	.95c	.41a	.68
	Mean	.93	.43	
Dwr	Low	.15c	.12b	.13
	High	.16d	.11a	.13
	Mean	.15	.12	
Tdw	Low	1.06b	.56a	.81
	High	1.11c	.53a	.82
	Mean	1.08	.54	
R	Mean	6.47	3.75	5.11
NA	Low	156.95b	120.78a	138.86
	High	202.57c	117.43a	160.00
	Mean	179.76	119.10	
g_c	Low	1.74a	1.73a	1.74
	High	2.46b	1.89a	2.17
	Mean	2.10	1.81	
g_m	Low	.602b	.430a	.516
	High	.755c	.411a	.583
	Mean	.679	.421	
WUE	Low			2.55
	High			2.42
	Mean	2.91	2.06	

[1/] For individual variables, if the interaction was significant, its means were examined by LSD-test, and the means marked by different letters are significantly different at 5% level by LSD-test.

substrate temperature resulted in smaller top, root, and total dry weights, but unfertilized seedlings showed the

opposite response to substrate temperature. Also, fertilized seedlings had lower NA, g_c, g_m under low than high substrate temperature. However, unfertilized seedlings showed no effect of substrate temperature on NA, g_c, or g_m.

As in experiment 1, upland seedlings had larger dry weights, and the difference between habitats was the greatest at site 3 (Table 4-8). The largest difference in WUE between habitats occurred for site 3 populations when fertilized (Table 4-9). However, no differences in WUE between habitats were statistically significant (Table 4-9).

TABLE 4-8. Top dry weight (Dwt), root dry weight (Dwr), and total dry weight (Tdw) by SITE x HABITAT

Variable	Habitat	Site 1	Site 2	Site 3	mean
Dwt (g)	Peatland	.69b	.71b	.58a[1/]	.66
	Upland	.72b	.69b	.68b	.70
Dwr (g)	peatland	.14	.14	.11	.13
	upland	.14	.14	.13	.14
Tdw (g)	Peatland	.83b	.85b	.69a	.79
	Upland	.86b	.83b	.81b	.84

[1/] For individual variables, if the interaction was significant, its means were examined by LSD-test, and the means marked by different letters are significantly different at 0.05% level by LSD-test.

TABLE 4-9. Water use efficiency (μmole CO_2/mmole H_2O) by SITE by HABITAT x FERTILIZATION (F)

	Site 1		Site 2		Site 3	
	Habitat					
F	peatland	upland	peatland	upland	peatland	upland
Yes	2.91bc	3.05c	2.87bc	2.85bc	2.91bc	2.69b[1/]
No	2.22a	2.03a	2.13a	2.07a	1.99a	2.12a

[1/] Means marked by different letters are significantly different at 0.05% level by LSD-test.

As in experiment 1, seedlings from site 3 were the smallest, but had the largest R (Table 4-10). In terms of dry weights and R, sites differed in their response to fertilization (Table 4-10). At site 3, fertilization had less effect on dry weight traits, but a larger effect on R than at the other sites.

TABLE 4-10. Height (Ht) by SITE, and top dry weight (Dwt), root dry weight (Dwr), total dry weight (Tdw), and DWT/DWR (R) by SITE x FERTILIZATION

Variable	Fertilization	Site 1	Site 2	Site 3
Ht (cm)	Mean	14.93b	14.78b	13.73a
Dwt (g)	Yes	.97a	.96a	.86b[1/]
	No	.44c	.44c	.40d
	Mean	.70	.70	.63
Dwr (g)	Yes	.16a	.16a	.13b
	No	.12c	.12c	.11d
	Mean	.14	.14	.12
Tdw (g)	Yes	1.13a	1.12a	.99b
	No	.56c	.57c	.51d
	Mean	.84	.84	.75
R	Yes	6.36a	6.19a	6.87b
	No	3.63c	3.75c	3.87c
	Mean	4.99	4.97	5.38

[1/] For individual variables, means marked by different letters are significantly different at 0.05% level by LSD-test.

Discriminant analysis results are summarized in Table 4-11. Separation was significant among the 6 populations, 3 sites, or between the 2 habitats on the basis of morphological traits, but not physiological traits. On the basis of morphological traits, however, the percentages of the grouped cases correctly classified were small (Table 4-11).

Based on morphology, populations from different habitats were not well grouped (Fig. 4-3). Upland and peatland populations at site 3 formed 2 clusters, while the rest of the populations formed another single cluster. Probably, the significant difference between habitats was due to the

difference between peatland and upland populations at site 3. Site 3 was the most different among the sites, and at this site habitats differed the most. Based on physiology, peatland populations were separated by function 1, and upland, by function 2; but populations from different habitats were not separated (Fig. 4-4). There was greater separation among peatland than among upland populations.

TABLE 4-11. Results of discriminant analyses for the data of morphology, and physiology

Source	function	% of variance	Canonical r	p value	Mean % of grouped cases correctly classified
Morphological data					
6 populations	1	78.26	.2670	.0000	25.37
	2	17.43	.1297	.0673	
	3	2.98	.0539	.6789	
	4	1.33	.0361	.5400	
2 habitats	1	100.00	.1049	.0333	56.53
3 sites	1	95.45	.2615	.0000	43.16
	2	4.55	.0591	.3471	
Physiological data					
6 populations	1	50.36	.0962	.8892	21.33
	2	30.87	.0754	.8991	
	3	18.70	.0588	.8804	
	4	0.08	.0037	.9952	
2 habitats	1	100.00	.0711	.4776	52.31
3 sites	1	94.59	.0581	.9635	
	2	5.41	.0139	.9876	36.31

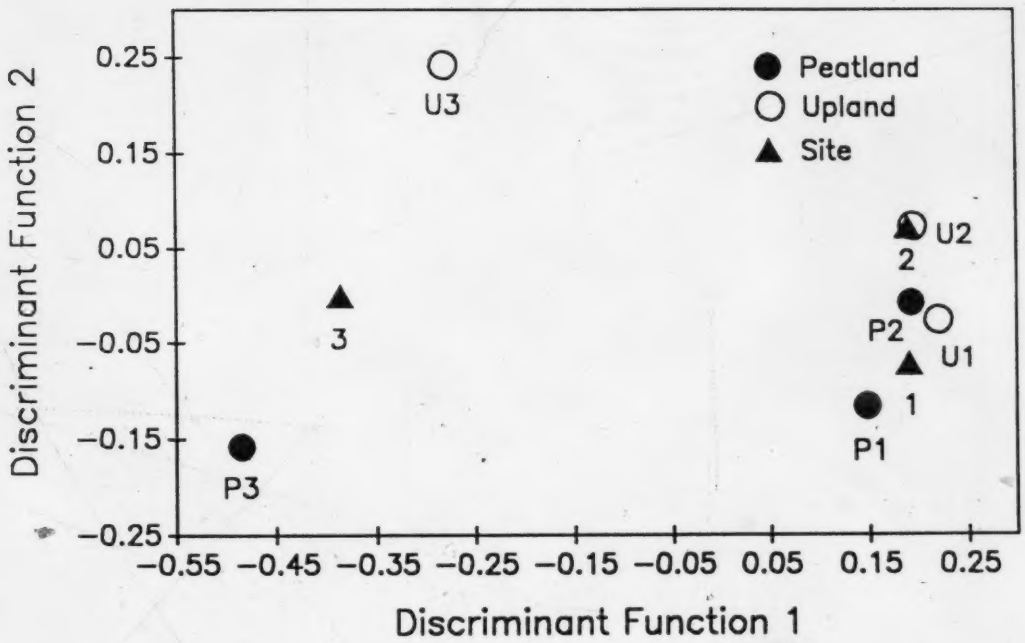

FIG. 4-3. Discriminant plot for the 6 black spruce populations at the 3 sites based on morphological data in experiment 2. Cases contained in P1, P2, P3, U1, U2, U3, and site 1, 2, and 3 were 1060 (212 seedlings x 5 variables, height, and top, root dry weight, total dry weight, and top/root dry weight), 800 (160 x 5), 790 (158 x 5), 800 (160 x 5), 790 (158 x 5), 775 (155 x 5), 1595 (319 x 5), 1590 (318 x 5), and 1565 (313 x 5) respectively.

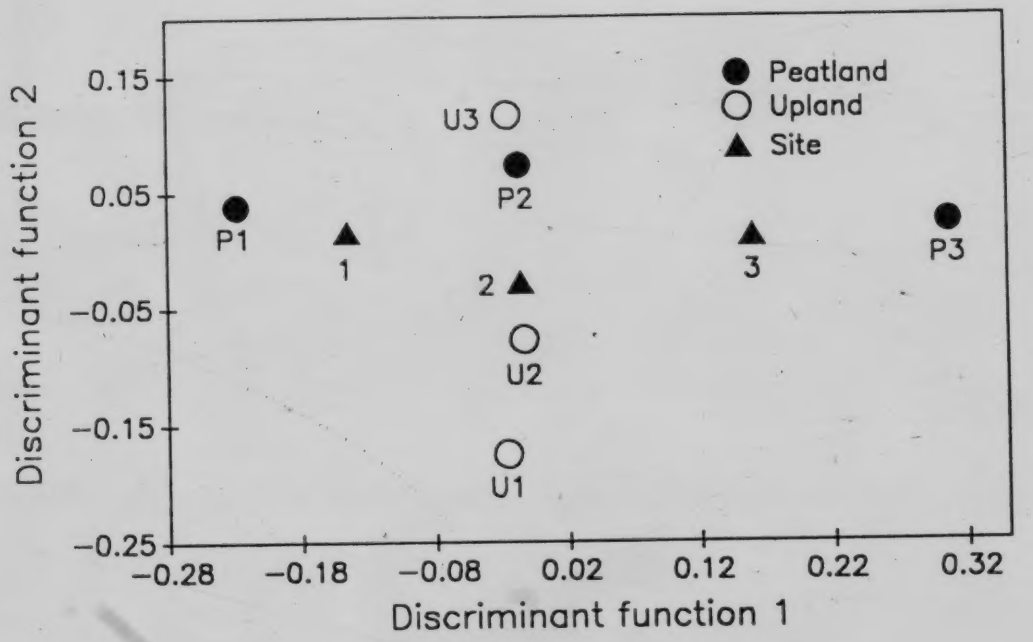

FIG. 4-4. Discriminant plot for the 6 black spruce populations at the 3 sites based on physiological data in experiment 2. Cases contained in P1, P2, P3, U1, U2, and U3 were 256 (64 seedlings x 4 variables, net assimilation, stomatal and mesophyll conductances to CO_2, and water use efficiency) respectively. Each site had 2 populations.

Within population genetic variation

In both experiments, there was no difference between the 2 habitats or among the 3 sites for any traits in mean genetic variation within populations (Table 4-12, 4-13). Discriminant analyses showed no significant separation among the sites or between the habitats based on within population genetic variation of the different traits (Table 4-14). However, site 3 was the most different among sites in both experiments (Fig. 4-5, 4-6). In the discriminant plot (Fig. 4-6), function 1 mainly separates site 3 from sites 1 and 2, function 2 mainly separates site 1 from site 2, and the 3 sites seem separated equally. However, the difference between site 3 and sites 1 and 2 was much larger than that between site 1 and 2 since the variance accounted for by function 2 was only about 1/7 of that by function 1 (Table 4-14).

Genetic variation among populations within habitats

The CV of population means (across families and treatments) for each habitat gives a measure of the extent of genetic variation among populations within habitat types. Although these values could not be compared statistically, the results are interesting (Table 4-12, 4-13). For 6 traits (Ht, Dwt, Dwr, Tdw, NA, and g_m), the peatland habitat showed greater variation among populations than the upland in both experiments. For R and g_c, the upland habitat showed greater among-population variation than the peatland in both experiments. For WUE, peatland habitat had higher among-population variation in experiment 1 (Table 4-12) and lower, in experiment 2 (Table 4-13) than upland. Overall, peatland populations were more variable than upland ones as they had larger among population genetic variation on 77% of the traits in experiment 1 and 67% in experiment 2.

Population variation in plasticity

There was a significant difference in plasticity between habitats for 5 traits in experiment 1 (Table 4-12). Peatland populations had higher plasticity in Ht, Dwt, Dwr, and Tdw, and lower, in g_c than upland populations. In experiment 2, there were no significant differences in mean population plasticity between peatland and upland habitats for any traits (Table 4-13). In both experiments, there were no significant differences in mean population plasticity among the 3 sites in any traits except WUE in experiment 2 (Table 4-12, 4-13). In experiment 2, site 3 had significantly higher plasticity of WUE (Table 4-13).

In discriminant analyses based on amount of plasticity for both experiments, the difference between peatland and upland habitats was significant, but the 3 sites did not differ (Table 4-14). Site 2 is the most different among sites in experiment 1, (Fig. 4-5); and site 3, in experiment 2 (Fig. 4-6).

TABLE 4-12. Means and P-values for data of plasticity and genetic variation within population, and genetic variation among populations within habitats for variables: height (Ht), top dry weight (Dwt), root dry weight (Dwr), total dry weight (Tdw), Dwt/Dwr (R), net assimilation (NA), stomatal and mesophyll conductance to CO2 (g_c and g_m), and water use efficiency (WUE) by SITE or HABITAT in experiment 1

Source	Ht	Dwt	Dwr	Tdw	R	NA	g_c	g_m	WUE
Genetic variation within populations									
Peatland	10.6	22.1	23.9	21.7	9.8	89.7	128.5	103.1	148.9
Upland	14.2	22.2	24.6	22.2	9.0	83.2	133.8	95.4	145.0
P-value	.056	.987	.854	.849	.613	.121	.053	.085	.074
Site 1	12.1	22.8	24.7	22.5	9.6	87.7	132.1	100.5	147.4
2	14.1	23.4	26.0	23.4	9.3	88.3	129.6	102.2	146.2
3	11.1	20.3	22.1	19.9	9.3	83.4	131.8	95.3	147.3
P-value	.597	.348	.673	.439	.989	.688	.833	.550	.923
Genetic variation among populations within habitats									
Peatland	8.24	13.42	17.65	14.08	1.97	9.26	3.13	10.84	6.34
Upland	4.65	8.70	16.40	9.96	5.35	2.31	4.23	2.10	3.20
Plasticity									
Peatland	63.1	131.0	129.0	130.4	33.0	87.6	150.7	104.0	168.1
Upland	57.3	126.9	123.7	126.1	33.7	81.2	153.9	98.2	167.4
P-value	.004	.001	.010	.001	.658	.200	.001	.350	.365
Site 1	59.8	129.3	127.5	128.9	32.8	84.3	152.4	101.1	167.6
2	60.0	129.2	125.1	128.2	33.7	88.5	152.0	106.9	167.2
3	60.7	128.4	126.5	127.8	33.6	80.9	152.6	95.4	168.4
P-value	.974	.949	.834	.941	.876	.462	.971	.286	.428

TABLE 4-13. Means and P-values for data of plasticity and genetic variation within population, and genetic variation among populations within habitats for variables: height (Ht), top dry weight (Dwt), root dry weight (Dwr), total dry weight (Tdw), Dwt/Dwr (R), net assimilation (NA), stomatal and mesophyll conductance to CO_2 (g_c and g_m), and water use efficiency (WUE) by SITE or HABITAT in experiment 2

Source	Ht	Dwt	Dwr	Tdw	R	NA	g_c	g_m	WUE
Genetic variation within population									
Peatland	9.0	11.2	14.7	11.1	11.7	77.1	92.8	78.5	80.9
Upland	7.0	13.0	13.2	12.3	12.4	76.0	92.7	77.5	82.5
P-value	.181	.378	.362	.500	.638	.169	.856	.290	.419
Site 1	7.0	12.8	14.1	12.4	11.4	76.5	93.1	77.5	80.0
2	7.8	12.0	13.1	11.5	12.6	76.2	92.9	77.5	81.3
3	9.3	11.5	14.7	11.3	12.2	77.0	92.3	79.1	83.9
P-value	.535	.894	.751	.892	.812	.734	.737	.189	.194
Genetic variation among populations within habitats									
Peatland	5.4	10.6	13.3	11.0	3.3	1.1	1.5	1.2	2.2
Upland	3.9	3.0	4.2	3.0	6.7	0.7	2.1	0.7	3.3
Plasticity									
Peatland	19.3	44.0	17.7	39.5	29.1	79.5	131.1	84.5	121.8
Upland	17.3	42.4	14.0	37.7	32.9	80.5	130.8	85.2	122.1
P-value	.115	.412	.312	.302	.210	.535	.884	.409	.792
Site 1	17.7	44.2	16.7	39.5	31.7	81.6	131.3	85.8	120.6
2	18.3	44.9	17.9	38.5	29.0	79.4	130.9	84.4	121.8
3	18.9	42.5	13.0	37.8	32.1	78.9	130.8	84.4	123.5
P-value	.796	.787	.567	.809	.717	.301	.976	.224	.044

TABLE 4-14. Results of discriminant analyses for the data of plasticity and genetic variation within population

Source	function	% of variance	Canonical r	p value	Mean % of grouped cases correctly classified
Experiment 1					
For data of plasticity					
3 sites	1	93.60	.9397	.5378	100.00
	2	6.40	.5775	.6665	
2 habitats	1	100.00	.9962	.0445	100.00
For data of variation within populations					
3 sites	1	97.26	.8893	.7661	83.33
	2	2.74	.3102	.9038	
2 habitats	1	100.00	.9759	.1928	100.00
Experiment 2					
For data of plasticity					
3 sites	1	96.38	.9666	.3898	83.33
	2	3.62	.5905	.6513	
2 habitats	1	100.00	.9993	.0102	100.00
For data of Variation within population					
3 sites	1	87.85	.7285	.9368	50.00
	2	12.15	.3678	.8648	
2 habitats	1	100.00	.8105	.7101	83.33

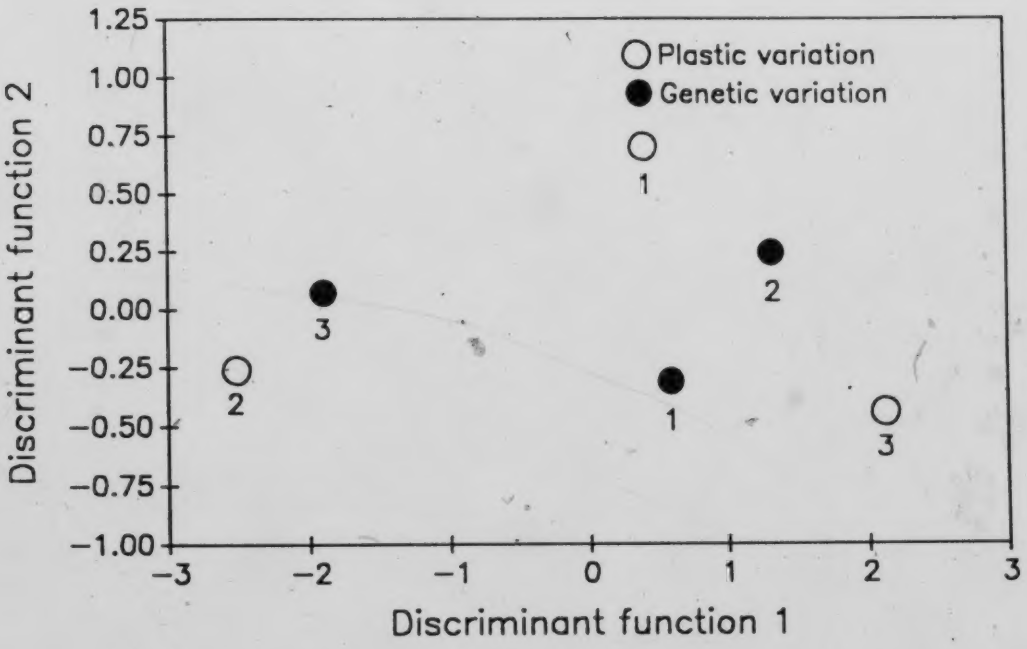

FIG. 4-5. Discriminant plot for the 3 sites based on genetic and plasticity components of variance in experiment 1. Each site contained 18 cases (2 populations x 9 variables, height, and top, root dry weight, total dry weight, and top/root dry weight, net assimilation, stomatal and mesohyll conductances to CO_2, and water use efficiency).

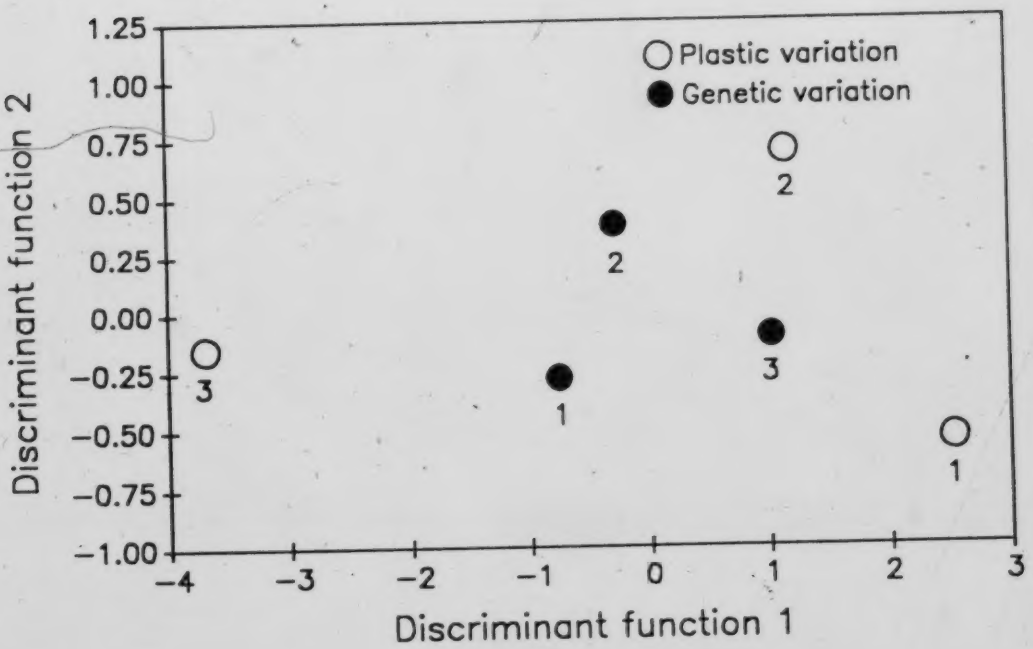

FIG. 4-6. Discriminant plot for the 3 sites based on genetic and plastic components of variance in experiment 2. Each site contained 18 cases (2 populations x 9 variables, height, and top, root dry weight, total dry weight, and top/root dry weight, net assimilation, stomatal and mesohyll conductances to CO_2, and water use efficiency).

4.4. Discussion

Treatment effects

As expected, seedlings grew larger at full light, medium moisture, and with fertilization. However, different substrate temperatures did not affect seedling growth much. Fowells (1965) suggested that, although shade-tolerant, black spruce develops better in full than in reduced light. My results support this conclusion.

Compared to upland, peatland black spruce trees are stunted, and rarely reach merchantable size (Lieffers and Rothwell 1986). The poor growth of the trees is attributed to the low substrate temperature, lower nutrient availability, and high water table (Van Cleve et al. 1981, Lieffers and Rothwell 1986) of peatlands. My results partially support this as watering every day (wet) (in experiment 1) or without fertilization (in experiment 2) reduced seedling growth. Also without fertilization, net assimilation (NA), and stomatal and mesophyll conductances to CO_2 (g_c and g_m) decreased.

Unexpectedly, physiological variables were negatively correlated with growth parameters (Table 4-2): higher NA, g_c, and g_m were associated with smaller seedling size when comparing seedlings watered every day with those watered every 5 or 10 days. This lack of concordance between these two set of traits might be explained by varying water relations. At the time of measurement, seedlings watered every 5 or 10 days may have been drought-stressed, while those watered every day could be constantly flood-stressed. However, the moisture conditions after watering and before drought stress became limiting may have been better for trees watered every 5 or 10 days than for those watered every day.

The cold substrate substantially reduced NA, g_c, and g_m. Low soil temperature has also been found to reduce NA, g_c, and g_m in other trees (Babalola et al. 1968, Turner and Jarvis 1975, Lawrence and Oechel 1983, Delucia 1986, Delucia and Smith 1987, Jurik et al. 1988). The reason for decreased g_c and g_m at low root temperature is water stress resulting from decreased water absorption due to both an increase in the viscosity of water and in root resistance (Kaufmann 1975, Turner and Jarvis 1975). Decreased g_c reduces CO_2 uptake by plants, and thus NA decreases. The nonstomatal reduction in NA by low soil temperature has been attributed to the reduction in electron transport capacity, the feedback inhibition of carbohydrate accumulation in needles, and the decrease in turgor of mesophyll cells (Delucia 1986), and low g_m (Turner and Jarvis 1975).

However, the cold substrate only slightly reduced seedling growth. This indicates that seedling growth is not simply affected by the physiological traits. In my study, at high substrate temperature, seedling NA was higher, but root

respiration may have been higher day and night than at low substrate temperature. Thus, the low NA at low substrate temperature might be compensated for by less respiration losses than at high substrate temperature. The net outcome would be a small effect of low substrate temperature on seedling growth and a poor correlation between NA and seedling growth.

Habitat differentiation

1. Adaptive responses to treatments

Ecotypic variation in plants is a well-known response to diverse environments (Stebbins 1950). In this manner, a species which grows in a wide range of environments adapts to a particular habitat (Kruckeberg 1967). Ecotypic differentiation has been demonstrated in trees in response to edaphic (Squillace and Bingham 1958, Kruckeberg 1967, Teich and Holst 1974, Jenkinson 1977, Furnier and Adams 1986, Millar 1989) and other environmental conditions (Pauley and Perry 1954, Vaartaja 1959, Hall 1955, Hermann and Lavender 1968). Peatland and upland habitats are very different in terms of abiotic (moisture, substrate temperature, light, and nutrient availability) and biotic (density, and species mixture) conditions. Ecotypes between the habitats have been demonstrated for white cedar (Habeck 1958, Musselman et al. 1975).

In my study, habitats showed some differential responses to environments, but not as expected, and/or their effects were site-dependent. If peatland and upland ecotypes exist in black spruce in response to the different abiotic conditions of the 2 habitats, trees should show adaptive responses to these aspects of the environment. However, in my study, habitats did not show adaptive responses to fertilization, light, or substrate temperatures (no significant interactions between HABITAT and LIGHT, FERTILIZATION, or SUBSTRATE TEMPERATURE). The interaction between HABITAT and MOISTURE was only significant on seedling height. However, upland seedlings were taller at more frequent watering than peatland seedlings. This is opposite to what was expected. In experiment 2, habitats differed in the way fertilization affected WUE, but the response varied among sites. In both experiments, peatland and upland populations were not well separated in discriminant plots based on morphological or physiological traits. Although the separation between habitats was significant based on morphological traits in experiment 2, the effect seemed due to the large difference between habitats at site 3, and only 56% of the grouped cases were correctly classified.

Thus, my results in this study support the conclusions by Fowler and Mullin (1977) and Parker et al. (1983) that there is little ecotypic differentiation in black spruce from

peatland and upland habitats.

A few explanations are possible for the lack of ecotypic differentiation between peatland and upland black spruce populations. Selection is expected to be severe during seed germination and early establishment when temperature and moisture are most critical. However, both peatland and upland stands originate after fire (Fowells 1965). In this situation, the seed bed conditions for germination may not be very different between the habitats. Also, seeds are likely to germinate during the moist period (early spring) when there may be no moisture difference between habitats. Furthermore, successful germination and establishment may also be determined by 'safe sites' (Harper 1977) which may not vary much between habitats with respect to selection pressure. Thus, the driving force for ecotypic differentiation is reduced.

While I hypothesized that upland black spruce trees would be better adapted to drought, drought resistant genotypes could be selected for in peatlands during the dry season (Denyer and Riley 1964). Likewise, the upper peatland surfaces may exhibit high temperature during these periods (R. Rothwell, unpublished). Thus, heat resistant genotypes might be favored in peatlands as in uplands.

Black spruce is a shade tolerant species whose seedlings can develop in the understorey with as little as 10% of full light (Fowells 1965). Thus, although competition for light is high in upland and nil in peatland populations, this factor may not be an important agent of selection in black spruce. Therefore, little differential response between peatland and upland black spruce trees to light conditions would be expected. While competing for light aboveground, upland black spruce trees might also face strong competition for nutrients and moisture below-ground as upland stands are usually dense and black spruce is often outgrown by other species. Thus, in upland sites selection might favor individuals that can do well with limited nutrient and moisture supply just as in peatlands.

Black spruce is an outcrossing species, and gene exchange between adjacent populations should be present. If gene exchange offsets selection pressure, little or no ecotypic differentiation would appear between peatland and upland populations. Finally, the experimental design may not be appropriate to detect the habitat differentiation or the differentiation was too weak to be detected.

The lack of differential adaptation responses for peatland and upland black spruce populations does not indicate, however, that the habitats are exactly the same. In both experiments, whenever the effects of habitat (including interactions with other factors) were significant on morphological traits, upland seedlings were larger. Morgenstern (1969a) also found black spruce seedlings of seed from dry sites were more vigorous than those from wet

sites. It has been reported that differences in inherent growth rate exist among progenies of white pine from different areas, with progenies from better sites growing more rapidly than those from poor sites (Squillace and Bingham 1958). Upland sites are more fertile and productive than peatlands. The faster growth of upland seedlings might reflect selection for faster growth due to more severe competition in upland populations. It could also be that the faster growing trees became the dominants or codominants which were selected for seed material in this study.

The difference in seedling growth between peatland and upland populations might be also due to maternal effects. Preconditioning of seed by climate and soil affects subsequent germination behavour and plant development (Rowe 1964). It has been shown that soil fertility influences seed composition, including the content of nitrogen, phosphorus, potassium, and magnesium (Youngberg 1952). There is a greater availability of nutrients in uplands than in peatlands. Thus, although the seeds used were similar in age and from a small weight range to reduce maternal effects on seedling performance, upland trees may have had better nutrient status, and so yielded higher quality seeds which subsequently produced faster growing seedlings.

2. Variation within populations

Variation within population indicates differences among families. Micro-environments, establishing gene pool, later recruitment, selection, mating systems, etc., could influence within population variation. Others have found that the genetic variability among peatland trees is higher than among upland trees (Parker et al. 1983, O'Reilly et al. 1985). However, results of my study do not support this conclusion. There was no significant difference in within-population genetic variation between habitats by ANOVA or discriminant analyses in both experiments. The discrepancies between my results and those in the above two might be due to differences in populations used and characters measured.

3. Variation in plasticity

In discriminant analyses, habitats were significantly different in terms of their plasticity. Peatland populations had higher plasticity than upland in seedling height (Ht) and dry weight traits, but lower in g_c in experiment 1. Although not significant, they also had higher plasticity than upland in Ht and dry weight traits in experiment 2. We can speculate about the consistently higher plasticity of peatland populations in both experiments for most of the morphological traits.

Plants, being immobile, are often exposed to environmental fluctuations which they are unable to avoid by behavioral means or homeostatic mechanisms (Bradshaw 1974). They adapt to the changing environments by means of genetic variation

or phenotypic plasticity (Marshall and Jain 1968) or by both (Scheiner and Goodnight 1984). Although there is considerable evidence that plasticity may provide an adaptive advantage to individuals in unpredictable habitats (see Macdonald 1988), amount of plasticity is not always correlated with environmental uncertainty (Scheiner and Goodnight 1984). Furthermore, natural selection might be expected to favor individuals that experience the least depression from their maximum potential fitness over the greatest range of suboptimal conditions. Thus, these individuals may be expected to display the least plastic response to environmental variability in performance characters (e.g., size or biomass) which estimate fitness (Taylor and Aarssen 1988).

Therefore, low plasticity of a population might develop in fluctuating environments as individuals which can do equally well in a wide range of environments would be favored. This may be one of the possible reasons for the low plasticity of upland populations as the conditions in uplands may be more fluctuating. In this respect, two of the possible factors might be light and leaf temperature. Black spruce trees are usually overtopped by other species in upland. Light conditions would vary with time of day for them in the stand because gaps and shifting sun-flecks would result in strong temporal fluctuations in light intensity and so in leaf temperature. But, this would not happen as much to trees in peatland as the stands are usually open. Another factor might be soil temperature. Peatland soil has a high water table and wetter soil with a higher heat capacity than upland soil. Thus, the soil temperature would fluctuate more both daily and seasonally in upland than in peatland soil.

Alternatively, low plasticity of upland may be due to maternal effects. As upland trees grow in better conditions producing seeds with higher vigor than peatland trees, their seedlings would be expected to perform more uniformly in a wide range of environments. Another possibility is that peatland is more stressful for the growth of black spruce trees than upland (Fowells 1965). The high plasticity of peatland trees might indicate a strategy for success or habitat specialization rather than an adaptive response (Taylor and Aarssen 1988).

Variation among sites

Differentiation between habitats varied with sites. This may suggest that genetic make-up and/or maternal influence between habitats were different among sites. Generally, the greatest difference between habitats was at site 3 in morphological and physiological traits. This may be due to the fact that the environmental differences between habitats were the greatest at site 3, thus resulting in the largest genetic and/or maternal differences between the peatland and upland populations at that site.

In many studies of trees, geographical distance has been correlated with population differentiation (e.g., Bousquet et al. 1987, Millar et al. 1988). Results of my study support this general conclusion. The physical distance between site 3 and sites 1 and 2 is about 6 times that between sites 1 and 2. Whenever the effects of SITE (including their interactions with other factors) were significant on any traits, site 3 was the most different. This was also illustrated by discriminant analyses. The reasons for this are not clear, but it may be due to locally site-specific selection, mating systems, maternal effects, reduced gene flow among distant populations, genetic drift, founder effects, differences in variation between habitats, or some combination of these.

Variation among populations within habitats

It has been reported that variation among peatland populations is less than among upland ones in terms of allozymes (O'Reilly et al. 1985). However, my results indicated that there was a greater variation among peatland than among upland populations for morphological and physiological traits. The reason for this may be that the environmental differences were greater among peatland than among upland sites resulting in greater variation in genetic make-up and/or maternal effects (seed quality) among peatland than among upland populations.

4.5. Conclusion

It was found that low light, nutrient stress (without fertilization), and water stress (wet or dry), reduced seedling growth. As moisture decreased or at low light, NA, g_c, g_m and WUE decreased. When fertilized, seedlings were smaller and had lower NA, g_c, and g_m at low than at high substrate temperature. Results showed no adaptive ecotypic differentiation in black spruce between the 2 habitats. However, upland seedlings were larger than peatland ones, and upland populations had lower plasticity. Within habitats, peatland populations were more variable than upland ones. Among sites, site 3 was the most different. The variation patterns between habitats, among sites, and among populations within habitats were attributed to natural selection, genetic effects, and/or maternal influences.

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Chapter 5. General discussion

5.1. Discussion

Differentiation in black spruce from peatland and upland habitats in Ontario was not well developed in terms of morphological traits (Fowler and Mullin 1977, Parker et al. 1983) or allozymes (Parker et al. 1983, Boyle et al. 1990). My results indicate that this is the case in peatland and upland black spruce in Alberta. In my allozyme study, there was little genetic differentiation between the habitats. In seed germination ecology, and seedling morphology and physiology, habitats did not show any adaptive responses to different environments. The lack of ecotypic differentiation in black spruce from the two habitats may be attributed to the extensive gene flow among populations and weak selection pressure differences between the habitats.

However, peatland and upland black spruce are not identical. The results showed that seeds from upland black spruce trees germinated better and their seedlings grew faster than those from peatland. Also, there were significant site-dependent habitat differences in seed germination, and seedling morphology and physiology. Furthermore, peatland populations had higher plasticity than upland for morphological traits.

Genetic variation in allozymes is generally consistent with that of phenotypic traits in trees (Guries 1984). However, there are many studies showing great variation among populations in phenotypic traits and little in allozymes (e.g., Cheliak et al. 1988, Farmer et al. 1988, Loopstra and Adams 1989, Moran and Adams 1989). In my study, although habitats showed little ecotypic differentiation in allozymes and phenotypic traits, the habitats did differ in terms of their morphological plasticity, and peatland populations had higher phenotypic plasticity than upland. Also the variation patterns among populations varied with sets of data. The allozyme study showed greater variation among upland than among peatland populations, but the opposite was shown for phenotypic traits. The greatest difference between peatland and upland habitats was at site 2 in allozyme study, but it was at site 3 in phenotypic traits. Among populations, the most different was upland population at site 3 in allozyme study, but it was peatland population at the same site in phenotypic traits. The lack of concordance in variation among different sets of characters indicates that different traits are controlled by different sets of genes or gene complexes of the genome (Linhart et al. 1989). It has also been shown that variability in one set of traits is not a good predictor of that in another (Linhart et al. 1989), and that it is more difficult to detect population variation in allozymes than in metric traits (Lewontin 1984). Thus, to understand variation among populations completely, traits should be

used together.

Among sites, site 3 was the most different in allozymes, seed germination, and seedling morphology and physiology. This seems related to geographical separation as the distance between site 3 and sites 1 and 2 is about 6 times of that between site 1 and 2. It is commonly found that geographical separation is correlated with population differentiation (e.g., Farmer and Reinhalt 1986, Fowler et al. 1988, Rehfelt 1988, Loopstra and Adams 1989, Cheliak et al. 1988, Schuster et al 1989, Lagercrantz and Ryman 1990). The effects of the separation have often been attributed to selection, random drift, founder effects, restricted gene flow among distant populations, and some combination of these. Besides these genetic effects, maternal effects may have also contributed to the variation pattern among sites in my study as seeds from natural populations were used. When such seeds are used in genetic studies, genetic and maternal effects can not be separated completely, thus great care should be taken when interpreting results.

5.2. Practical applications

To obtain optimum growth of black spruce in reforestation or in seedling production, treatments (e.g., site preparation, control of competition from other species, irrigation, and fertilization) should be made to provide sufficient light, and adequate soil moisture and nutrients.

Although peatland and upland habitats were not well differentiated, showing no adaptive responses to treatments as expected in my studies, it does not mean that upland and peatland populations are identical ecologically. The faster growth of upland seedlings has great relevance in terms of reforestation. It is recommended that, to reforest productive uplands, seeds from upland trees should be used. However, the use of upland seeds to reforest peatlands is not recommended because the better growth of upland seedlings in my greenhouse study may not be realized in peatlands in nature. Further study utilizing field reciprocal transplanting would provide essential information in this respect. When using seeds from peatland trees to reforest peatlands, care should be taken to ensure to obtain the best sources as variation among peatland populations is large. To do so, it is necesary to match the environmental conditions of the seed source populations with those of the reforestation sites, and among these potential populations, collect seeds from those populations in which trees grow the best.

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6. Appendices

Appendix 1. Means for variables, height (Ht, cm), top dry weight (Dwt, g), root dry weight (Dwr, g), total dry weight (Tdw, g), dwt/dwr (R), net assimilation (NA, μmole $CO_2 g^{-1} h^{-1}$), stomatal and mesophyll conductances to CO_2 (g_c and g_m, $mmole g^{-1} h^{-1}$), and water use efficiency (WUE, μmole CO_2/mmole H_2O) by Habitat by Site by Light by Moisture Regime (Moisture) (Wet-soaking once/day, Medium-soaking once/5 days, and Dry-soaking one/10 days) in experiment 1 of Chapter 4.

			Habitat					
			Peatland Site			Upland Site		
Variable	Light	Moisture	1	2	3	1	2	3
Ht	20%	Wet	5.69	5.59	4.84	6.14	5.94	5.99
		Medium	7.76	7.43	6.26	8.34	7.81	7.33
		Dry	6.13	5.89	5.48	6.78	6.11	6.29
	100%	Wet	7.93	8.28	6.58	9.64	9.23	8.25
		Medium	22.89	21.93	19.54	22.52	22.13	20.88
		Dry	17.68	17.11	15.69	17.47	16.47	15.72
Dwt	20%	Wet	.03	.03	.03	.03	.04	.04
		Medium	.05	.04	.03	.05	.05	.04
		Dry	.03	.03	.03	.04	.03	.03
	100%	Wet	.24	.24	.18	.33	.31	.25
		Medium	1.50	1.43	1.11	1.55	1.43	1.28
		Dry	1.01	.96	.82	1.00	.90	.87
Dwr	20%	Wet	.005	.004	.004	.005	.005	.004
		Medium	.005	.004	.004	.005	.005	.004
		Dry	.004	.003	.003	.004	.004	.004
	100%	Wet	.059	.058	.044	.085	.078	.058
		Medium	.289	.261	.201	.316	.253	.216
		Dry	.225	.228	.194	.236	.222	.209
Tdw	20%	Wet	.04	.04	.03	.04	.04	.04
		Medium	.05	.05	.04	.05	.05	.05
		Dry	.04	.03	.03	.04	.03	.04
	100%	Wet	.30	.30	.22	.42	.38	.31
		Medium	1.79	1.69	1.31	1.86	1.68	1.49
		Dry	1.24	1.19	1.01	1.24	1.12	1.08
R	20%	Wet	7.73	8.13	8.08	7.49	7.92	8.47
		Medium	9.31	10.12	9.39	9.34	9.22	10.11
		Dry	8.00	8.60	9.16	8.73	8.60	9.33
	100%	Wet	4.30	4.30	4.32	4.03	4.13	4.87
		Medium	5.80	5.98	5.77	5.33	6.38	6.42
		Dry	4.61	4.37	4.44	4.34	4.23	4.27

Appendix 1. *continued*

Variable	Light	Moisture	Habitat: Peatland Site 1	Peatland Site 2	Peatland Site 3	Upland Site 1	Upland Site 2	Upland Site 3
NA	20%	Wet	78.5	87.3	83.7	95.7	90.9	74.7
		Medium	58.7	54.1	51.5	49.4	46.6	56.1
		Dry	27.9	24.5	25.9	23.0	31.1	24.2
	100%	Wet	99.7	105.3	90.4	83.4	80.1	85.1
		Medium	78.9	92.1	68.9	62.7	67.1	73.1
		Dry	76.9	103.2	74.6	71.3	83.9	82.3
g_c	20%	Wet	1.35	1.31	1.41	1.27	1.18	1.31
		Medium	.95	.85	.85	.78	.89	.79
		Dry	.70	.58	.62	.62	.67	.50
	100%	Wet	2.04	2.16	2.12	1.93	1.99	1.81
		Medium	1.88	1.92	2.10	1.79	2.02	1.82
		Dry	1.82	1.76	2.00	1.54	1.72	1.59
g_m	20%	Wet	.309	.351	.347	.395	.381	.292
		Medium	.231	.216	.209	.198	.181	.249
		Dry	.108	.095	.101	.083	.117	.094
	100%	Wet	.398	.432	.351	.331	.302	.336
		Medium	.301	.437	.286	.282	.337	.314
		Dry	.306	.367	.277	.241	.252	.288
WUE	20%	Wet	.81	.90	.82	.81	.74	.88
		Medium	.77	.95	.58	.76	.74	.85
		Dry	.72	.88	.62	.77	.66	.79
	100%	Wet	1.10	1.25	1.11	1.49	1.50	1.15
		Medium	1.37	1.23	1.36	1.30	1.27	1.65
		Dry	.88	.92	.95	.77	.96	.93

Appendix 2. Means for variables, height (Ht, cm), top dry weight (Dwt, g), root dry weight (Dwr, g), total dry weight (Tdw, g), dwt/dwr (R), net assimilation (NA, μmole $CO_2 g^{-1} h^{-1}$), stomatal and mesophyll conductance to CO_2 (g_c and g_m, $mmole g^{-1} h^{-1}$), and water use efficiency (WUE, μmole CO_2/mmole H_2O) by Habitat by Site by Substrate Temperature (Temp) (high: room temperature, and low: 5°C at 5 cm from the peat surface) by Fertilization (Fert) in experiment 2 of chapter 4.

			Habitat					
			Peatland Site			upland site		
Variable	Fert	Temp	1	2	3	1	2	3
Ht	Yes	High	17.29	17.71	16.40	17.44	17.29	16.43
		Low	17.20	16.91	15.47	16.83	16.44	15.38
	No	High	12.79	12.43	11.31	13.35	12.72	12.28
		Low	12.07	12.86	10.90	12.48	11.88	11.52
Tdw	Yes	High	1.10	1.07	.91	1.14	1.09	1.05
		Low	1.15	1.19	.93	1.14	1.15	1.08
	No	High	.56	.55	.47	.61	.59	.58
		Low	.51	.61	.43	.56	.51	.54
Dwt	Yes	High	.95	.91	.79	.98	.94	.92
		Low	.98	1.02	.80	.98	.97	.94
	No	High	.43	.44	.37	.47	.46	.46
		Low	.39	.48	.34	.44	.40	.43
Dwr	Yes	High	.15	.16	.13	.16	.15	.13
		Low	.17	.17	.13	.16	.18	.14
	No	High	.12	.12	.10	.13	.13	.12
		Low	.11	.13	.09	.12	.11	.11
R	Yes	High	6.48	6.10	6.64	6.39	6.72	7.13
		Low	6.22	6.19	6.63	6.34	5.75	7.11
	No	High	3.60	3.92	3.92	3.61	3.53	3.90
		Low	3.53	4.03	3.87	3.75	3.49	3.80

Appendix 2. *continued*

Variable	Fert	Temp	Habitat: Peatland Site 1	Peatland Site 2	Peatland Site 3	upland site 1	upland site 2	upland site 3
NA	Yes	High	205.2	198.6	202.7	210.3	203.2	195.3
		Low	153.7	157.4	154.6	154.0	161.5	160.1
	No	High	116.5	118.5	123.7	113.4	119.9	120.5
		Low	129.9	117.1	117.6	114.4	122.4	123.2
g_c	Yes	High	2.45	2.51	2.35	2.48	2.53	2.41
		Low	1.71	1.81	1.71	1.57	1.80	1.88
	No	High	1.81	1.95	2.08	1.74	1.84	1.91
		Low	1.75	1.65	1.80	1.82	1.67	1.70
g_m	Yes	High	.752	.732	.767	.794	.756	.728
		Low	.586	.598	.603	.608	.624	.594
	No	High	.412	.415	.424	.402	.391	.422
		Low	.463	.422	.415	.405	.438	.438
WUE	Yes	High	2.92	2.98	2.90	2.94	2.76	2.71
		Low	2.97	2.85	2.91	3.28	3.02	2.72
	No	High	1.99	2.01	1.95	1.98	1.91	2.00
		Low	2.36	2.21	2.04	2.01	2.14	2.15